HYBRIDIZATION AMONG WESTERN WHIPTAIL LIZARDS (*CNEMIDOPHORUS TIGRIS*) IN SOUTHWESTERN NEW MEXICO: POPULATION GENETICS, MORPHOLOGY, AND ECOLOGY IN THREE CONTACT ZONES

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ABSTRACT

Cnemidophorus tigris punctilinealis of the Sonoran Desert and *C. t. marmoratus* of the Chihuahuan Desert contact each other and interbreed in the Animas Valley of southwestern New Mexico. More than 600 specimens have been examined from the contact region, and data on biochemical genetics (mitochondrial DNA haplotypes, protein electrophoresis of nuclear gene products), chromosomes, external morphology (coloration, size, scalation), reproduction, and fitness have been compared for three hybrid zones. Habitats in the contact region were mapped and photographed, and they are discussed in the context of vegetational changes during Pleistocene to Recent times, which affected the geographic distribution of these animals.

Data from mitochondrial DNA, allele frequencies at four protein loci (of 36 analyzed), and body coloration demonstrate that the areas of contact have steep, concordant, and coincident step-clines in which most gene exchange occurs in hybrid zones that are 3.2–7.8 km wide. Analyses of allele frequencies, genotype frequencies, and fixation indices (including Hardy-Weinberg equilibrium, linkage equilibrium, and cytonuclear equilibrium) indicate a population structure determined primarily by random mating and an absence of selection against hybrids. Estimates of gene flow indicate that the clines resulted from neutral secondary contact initiated with the newest reconnection of the Sonoran and Chihuahuan Deserts within the present interglacial episode, from 1000 to 5000 years ago. This timeframe is consistent with paleoecological data from packrat middens.

Analyses of karyotypes, morphology, reproduction, and physiology also fail to detect differences in fitness among lizards with various genotypes. Although it is possible that there are fitness differences that are too small to be detected by the sample sizes we employed, the data indicate that reproductive success, fitness, and the dynamics of populations within the hybrid zones presently are no different from those in nonhybrid populations. Earlier data, which suggested that one of the step-clines was moving, are not supported.

The clines are located in fragile semiarid habitats that are subject to desertification. Consequently, we present considerable data and dated photographs of habitats, precise locations of sampling sites, and local allele frequencies, so that future investigators can monitor changes in position, width, or dynamics of these hybrid zones.

In addition, the population genetics data are discussed in the context of the following: (1) absence of rare, apparently novel alleles forming in the hybrid zones; (2) genetic comparisons with additional subspecies of *C. tigris* (*C. t. aethiops* and *C. t. septentrionalis*); and (3) interspecific hybridization between *C. tigris* and other whiptail lizards of either bisexual or unisexual (parthenogenetic, clonal) species. *Cnemidophorus tigris* is one of the ancestors of some of the parthenogens, which are of hybrid origin, and our interest in their evolutionary history fuels our efforts to improve understanding of hybridization among whiptail lizards.

INTRODUCTION

Contact between morphologically recognizable congeneric populations may or may not result in interbreeding. In the absence or near absence of interbreeding, sympatry and syntopy may occur; the populations remain on different evolutionary tracks and unquestionably have diverged sufficiently from their common ancestor to be separate species. If interbreeding prevails, however, and F_1 hybrids and subsequent generation and backcross individuals are fertile, abundant, and represent the norm in contact regions, the specific or subspecific status of the populations may be contentious, while other aspects of their biology are very interesting (for general reviews, see Barton and Hewitt, 1985, 1989; J. Arnold, 1993; M. L. Arnold, 1997; Barton and Gale, 1993; Harrison, 1993; Patton, 1993; Patton and Smith, 1994).

Hybridization has intrigued biologists for decades. Dynamic interactions between differentiated populations provide opportunities to observe evolution in action and to ponder its consequences. Among the vertebrates in particular, hybridization between whiptail lizards offers special opportunities for gaining insights into these processes because the unisexual species of *Cnemidophorus* that reproduce by parthenogenetic cloning are probably all of hybrid origin (Vrijenhoek et

al., 1989; Dessauer and Cole, 1989; Cole and Dessauer, 1993, 1995). Detailed analyses of hybrid zones may offer insights into the origin of all-female species coinciding with the extinction of spermatozoa in unisexual lineages.

What is the evolutionary and ecological history of a hybrid zone—when and why did it form? Is hybridization equal in both directions, or is it a one-way affair, in which the females of one form mate with males of the other, but not vice versa? Do hybrids with various alternative combinations of genes have the same fitness and fecundity as nonhybrids? In time measured by hundreds or thousands of generations, will the populations gradually blend through a zone of intergradation, or will the hybrid zone remain narrow? If the hybrid zone remains narrow, is it a result of selection against hybrids, differences in immigration and genetic swamping in the vicinity of a population density sink, or is the contact so recent that genetic mingling has just begun?

How does one measure hybrid zones and the effects of hybridization? Are the changes in gene frequencies or clines of various independent characters concordant in shape and coincident in location? Does introgression occur for certain genes but not for others? Do new alleles originate most frequently in the hybrid zone, and what is their fate? Are the characteristics of one hybrid zone repeated at each point of contact of the taxa involved, or do separate contact zones have different interactions, and if so, why? These and related questions are addressed in this report.

Only a few hybrid zones among lizards have been investigated extensively (for example, see reviews in Dessauer and Cole, 1991; Sites et al., 1992, 1995, 1996; Reed and Sites, 1995; Dosselman et al., 1998). We are particularly interested in hybridization among whiptail lizards of the genus *Cnemidophorus* for three reasons: (1) the contact region discussed in this paper was first investigated more than 30 years ago (Zweifel, 1962; Dessauer et al., 1962), and we are now using improved multidisciplinary methods to compare the same hybrid zones at two points in time, a quarter of a century apart; (2) all-female parthenogenetic clones have originat-

ed independently perhaps 10 times from interspecific hybridization among bisexual forms of *Cnemidophorus* (reviewed by Dessauer and Cole, 1989; Cole and Dessauer, 1995; and to a limited extent by Wright, 1993), so we are investigating sex ratios, reproduction, and other aspects of hybrids; and (3) the process of horizontal gene transfer between lineages is relevant to understanding the systematics and phylogeny of *Cnemidophorus*, and has broader implications concerning speciation as well.

This report focuses on the western whiptail lizard, *Cnemidophorus tigris*, of the *tigris* species group (Lowe et al., 1970b). The number of species recognized in this group ranges from one (*Cnemidophorus tigris* sensu lato, with 12 or more subspecies) to six or more, depending on the authority. Wright (1993, 1994) favors the single polytypic species point of view, whereas others give specific status to certain recognizable populations (for example, see Walker et al., 1966; Walker and Maslin, 1981; Maslin and Secoy, 1986; Hendricks and Dixon, 1986; Taylor and Walker, 1991). Much of this taxonomic disagreement concerns populations restricted to islands in the Gulf of California (Sea of Cortez), but some is based on recognizable mainland and peninsular (Baja California) populations that interbreed in contact zones of various widths (for example, see Taylor, 1988, 1990; Dessauer and Cole, 1991; Taylor et al., 1994; see also the intriguing paper by Radtkey et al., 1997).

The *Cnemidophorus tigris* group occurs in western North America, usually at low elevations in arid and semiarid lands. The lizards occur primarily in desertscrub, so their overall range approximates that of the Great Basin, Mohave, Sonoran, and Chihuahuan Deserts (fig. 1), although they are not restricted to deserts throughout their range. Our primary focus herein concerns populations in southwestern New Mexico and southeastern Arizona (rectangle in fig. 1), where the desert-grassland, Sonoran Desert, and Chihuahuan Desert biota approach each other and intermingle (e.g., Bender, 1982; Brown, 1982a). This region is where Zweifel (1962) and Dessauer et al. (1962) first analyzed hybridization among *Cnemidophorus tigris gracilis* of the Sonoran Desert and *Cnemi-*

dophorus tigris marmoratus of the Chihuahuan Desert (fig. 1). The nomenclature applied to these taxa today is as follows: (1) *C. t. gracilis* is referred to by its senior synonym, *C. t. punctilinealis* (see Taylor and Walker, 1996); and (2) the name *C. t. marmoratus* is still used (Dessauer and Cole, 1991; also, see discussion under "Taxonomic Status of *C. t. marmoratus*"). We also provide comparative genetic data from populations outside the contact region, including the adjacent subspecies *C. t. septentrionalis* primarily of northern Arizona and Utah, as well as *C. t. aethiops* primarily of Sonora, Mexico (fig. 1). These comparisons suggest that the genetic distinctions between *punctilinealis* and *marmoratus* are not as great as one might think upon viewing the dramatic stepclines in the contact region.

Six additional species of *Cnemidophorus* (table 1) are syntopic with *C. tigris* within the contact region (fig. 1, rectangle). One of these, *C. inornatus*, is bisexual (dioecious, gonochoristic, with separate sexes), as is *C. tigris*, whereas the other five (table 1) are unisexual (all-female) species that reproduce by parthenogenetic cloning (Hardy and Cole, 1981; Dessauer and Cole, 1986).

Two of the unisexual species (table 1, *neomexicanus* and *tesselatus*) are daughter species that resulted from clonal reproduction of F_1 hybrids between *C. tigris marmoratus* and other species of *Cnemidophorus*. Consequently, haploid genomes of *C. tigris* in diploid and triploid interspecific hybrids can be very successful in ecological situations far removed from those experienced by *C. tigris* itself, as seen especially in *C. tesselatus* and also in *C. neotesselatus* in Colorado. In addition, *C. tigris* occasionally hybridizes with females of unisexual taxa (e.g., Lowe et al., 1970a; Cole, 1979; Hardy and Cole, 1998; Taylor et al., in prep.). Therefore, we also present new data and review rare instances of hybridization between *C. tigris* and most of these other species, both unisexual and bisexual, in the contact region. As recently emphasized by Hardy and Cole (1998), researchers should be aware that such hybrids can be easily misidentified in the field.

Hybridization in nature frequently occurs in ecotones or other areas of ecological change. A specific locality may be in transition at some periods in time but not in others (fig. 2). In a preliminary report (Dessauer and Cole, 1991), we suggested that one of the clines discussed here is moving significantly in space and time, on the order of one kilometer to the east in about 25 years, but our most recent analyses suggest this may not be so. We also suggested that this is not the first time these subspecies of *C. tigris* have contacted one another, but cycles of contact and separation have occurred with interglacial and glacial periods, respectively, for hundreds of thousands of years or more. The situation prevailing today reflects conditions of the interglacial period in which we live, with human-mediated changes superimposed on natural changes in climate and vegetation. Therefore, we extended our earlier comparison and document here not only details on the genetics and morphology of the lizards, as well as the nature of the hybrid zones over 25 years, but also the distribution of the relevant habitats as they existed during the time of this study. Consequently, we provide a detailed baseline against which future studies can be compared, perhaps in 100 years, but somebody else will have to do those studies.

The purposes of this report are as follows: (1) to examine, in the context of the whiptail lizards in the contact region (fig. 1, rectangle), the general phenomena of hybrid zones mentioned above; (2) to describe in detail, with the improved technology of the 1990s, the genetics (allozymes, mitochondrial DNA, and karyotypes), morphology (coloration, size, scalation, and reproductive organs), and local habitats and distribution of these lizards; (3) to expand (since the reports of 1962 and 1991) the geographic coverage in order to compare all three of the major independent hybrid zones within the contact region; (4) to discuss the origin and distribution of unique and uncommon alleles and the origin of parthenogenesis, both of which may be associated with hybridization; and (5) to provide predictions and a firm baseline of comparative data so that future analyses can assess changes in either or both the lizards and their desert and desert-grassland habitats through time.
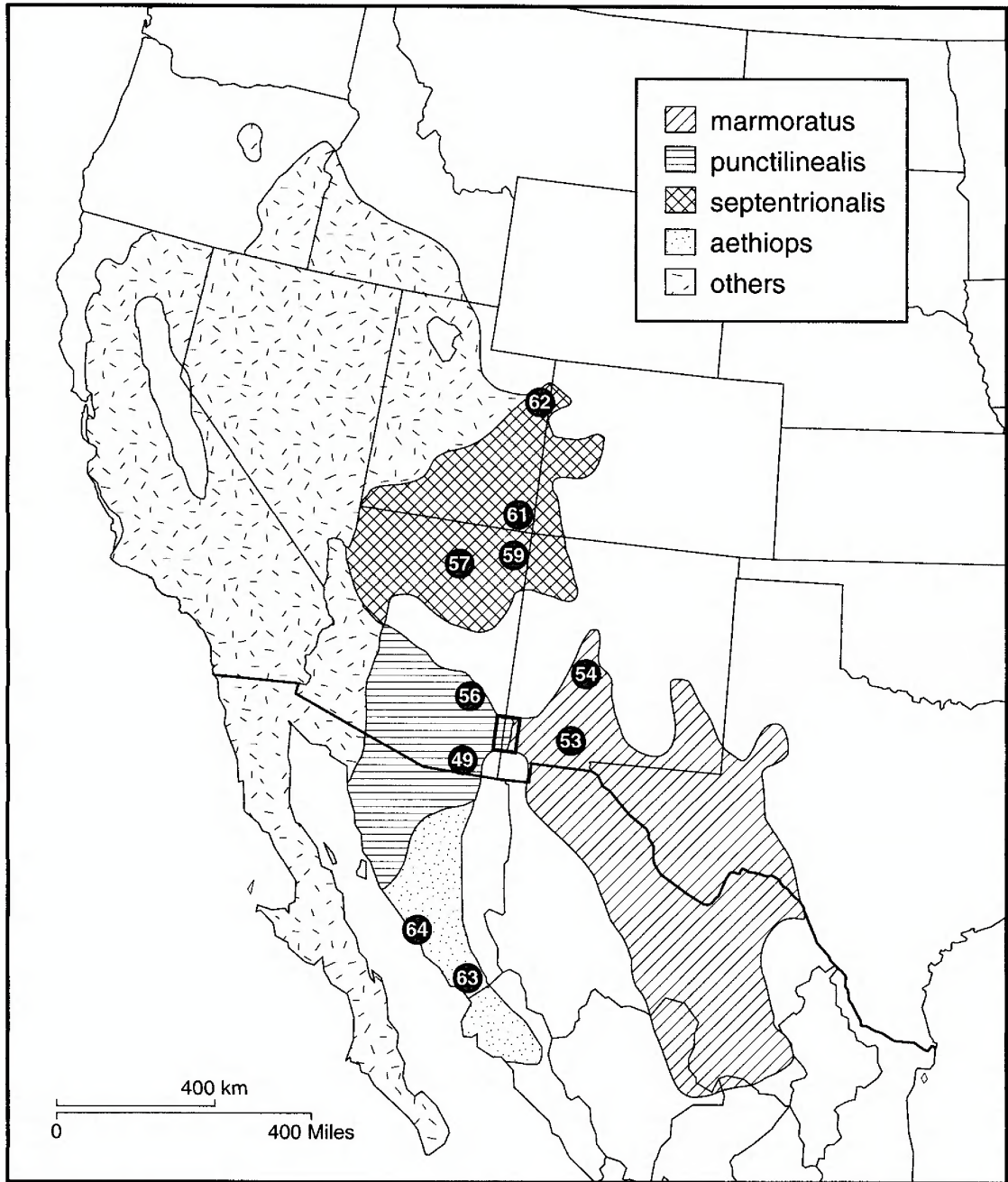
Fig. 1. Geographic range of the western whiptail lizard, *Cnemidophorus tigris* sensu lato, in the continental southwestern United States and northern Mexico. Rectangle (SE Arizona and SW New Mexico) outlines the contact region (detailed in figs. 3–5) where *C. t. punctilinealis* interbreeds with *C. t. marmoratus*. Numbers designate collecting sites (appendix 2) for specimens additional to those obtained within the contact region (fig. 3; appendix 1).

TABLE 1
The Seven Species of Whiptail Lizards That Live Within the Contact Region[a]

Species	Reproduction	Ploidy	*tigris* genome[b]	Hyb[c]	Preferred habitats[d]
C. inornatus	Bisexual	2n	—	Yes	Grassland
C. tigris	Bisexual	2n	—	—	Desertscrub
C. exsanguis	Unisexual	3n	No	No	Woodland + grassland
C. neomexicanus	Unisexual	2n	Yes	Yes	Desertscrub + grassland
C. sonorae	Unisexual	3n	No	Yes	Woodland + grassland
C. tesselatus[e]	Unisexual	2n	Yes	Yes	Desertscrub
C. uniparens	Unisexual	3n	No	No	Grassland + desertscrub

[a] The contact region is illustrated in figures 1 and 3.

[b] The unisexual species all had an origin involving cloning of an F_1 interspecific hybrid female. "Yes" indicates that *C. tigris* was one of the parents of that original hybrid, "no" indicates it was not.

[c] Does this taxon hybridize rarely with *C. tigris*? No F_1 hybrids with *C. inornatus* are known, but ancestry of *C. neomexicanus* was a hybrid between *C. tigris* × *C. inornatus*.

[d] These are the habitats occupied primarily in the contact region. Some of these species occur in other habitats elsewhere.

[e] Referred to as *C. dixoni* by many authors.

GENERAL METHODS

This report involves multidisciplinary research at various levels of biological organizaton. In the field, we collected specimens of lizards, determined precisely the site of collection with satellite technology, took habitat notes and photographs, and mapped vegetative communities. Most of this was accomplished during the summers from 1983 to 1993.

For biochemical analyses, we aimed for a minimum sample size of 10 lizards per collecting site (about 2–3 ha; see below), which may allow detecting allelic diversity at the 5% level of variation. We collected with nooses, drift fences, manual excavation of burrows, and .22 calibre dust shot. Tissue samples were either removed immediately and frozen in liquid nitrogen in the field, or the lizards were carried to the laboratory (dead lizards can be transported on ice for a few hours without losing tissue samples, as long as the blood is sampled immediately in the field). The following samples were frozen routinely: blood (centrifuged prior to freezing in order to separate plasma and packed cells), heart, liver, skeletal muscle, kidneys, and stomach and duodenum (after removal of any food remains, visible parasites, and the pancreas because of its potentially destructive enzymes). Color notes were made prior to preservation of the voucher specimen, and all notes and samples were cross-correlated for reference to the specific individual.

The database includes haplotypes of mitochondrial DNA (analyzed with allele-specific oligonucleotide probes), allozymes (analyzed by protein electrophoresis), karyotypes, and morphology (coloration, size, scalation, and internal anatomy). We used statistical techniques as appropriate to the different datasets and questions being addressed. The methods are detailed in relevant sections of the text.

SAMPLE SIZES AND LIZARD DENSITIES

Critical readers will note that we could have used more robust statistical tests and perhaps better resolved certain questions, if only we had significantly increased sample sizes. However, in biology there can be significant constraints imposed by natural population densities and the importance of conserving populations. How many specimens can be removed without causing a negative affect on local populations?

Population densities have not been estimated for either *C. t. punctilinealis* in Cochise County, Arizona, or for this taxon or *C. t. marmoratus* in Hidalgo County, New Mexico. The geographically closest populations studied suggest that for *punctilinealis* in central Arizona, there may be 13–36 in-
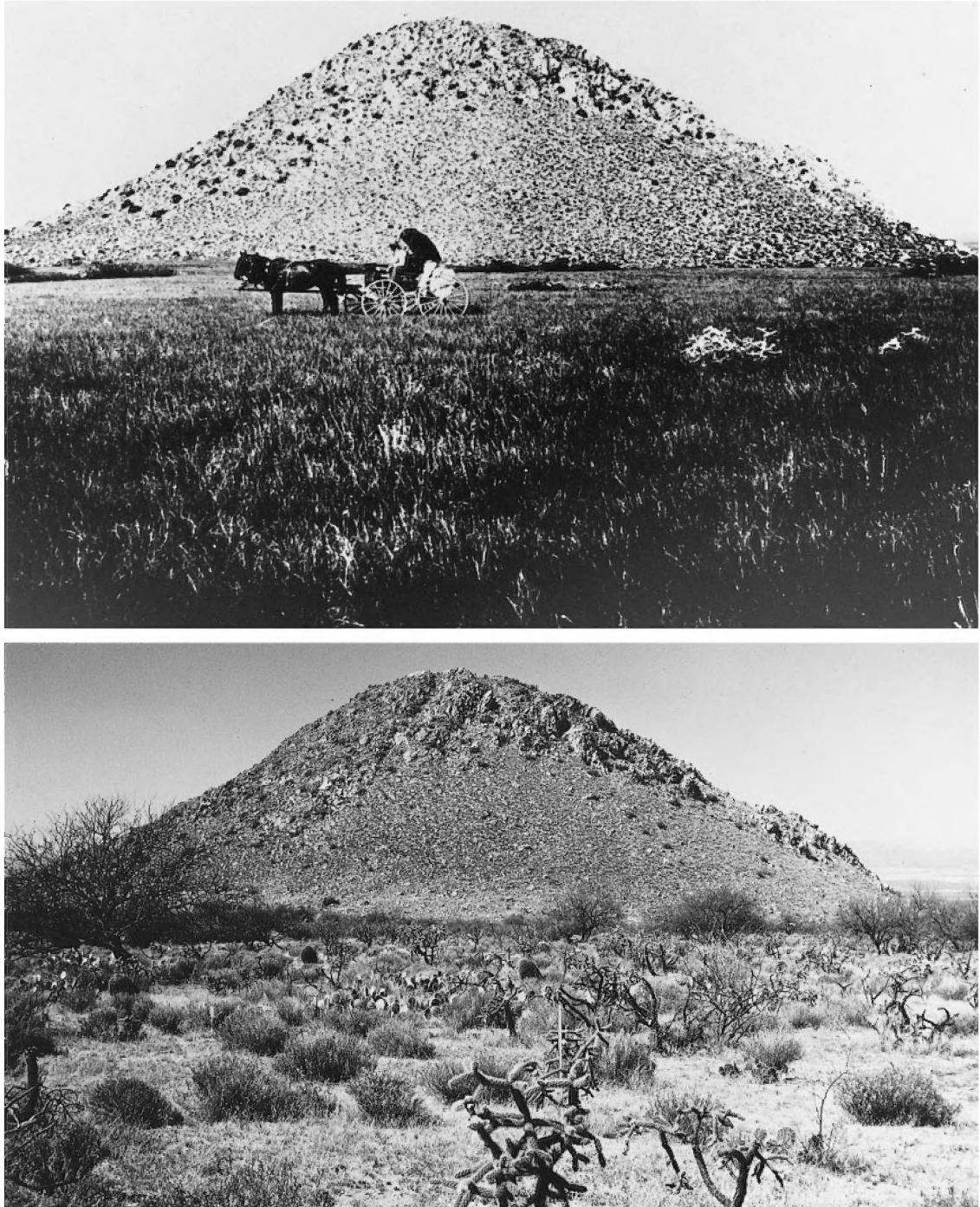
Fig. 2. Site 49 (appendix 2). Huerfano Butte (E side), Pima County, Arizona, about 43 km SSE Tucson, showing dramatic change in vegetation (desertification) in less than 70 years (based on Lowe et al., 1970a; their fig. 2). **Top**. About 1902, courtesy of Walter S. Phillips, University of Arizona. **Bottom**. 16 March 1969.

dividuals per hectare (Parker, 1972). For *marmoratus* in Dona Ana County, New Mexico, estimates were 8–34 individuals per hectare (Price et al., 1993) in one area, and, in another area, 40–70 individuals per hectare (Whitford and Creusere, 1977). The wide ranges in densities reflect in part fluctuating population sizes from year to year, depending on variation in rainfall and insect availability, as well as other factors, such as the uneven distribution of preferred microhabitats.

At most of our collecting sites, we took 10 specimens from approximately 2 or 3 ha. Considering the population density estimates for Dona Ana County (above), we might have removed from 7–62% of the deme in that sampling. However, these sites are within long, continuous stretches of similar habitat, not small, isolated patches. For a few sites from which we have larger samples, specimens were accumulated by repeated sampling over several to many years. Even so, sample sizes remain small from the perspective of statisticians and certain geneticists; this constraint is imposed upon us by nature.

ACKNOWLEDGMENTS

Fig. 3.   The contact region. Numbers designate collecting sites. Compare with figure 49.

THE CONTACT REGION
(*C. T. PUNCTILINEALIS* × *C. T. MARMORATUS*)

The vast majority of our specimens (607) were collected at 48 sites in an approximately 60 × 75 km area of Hidalgo County, New Mexico, and Cochise County, Arizona (the contact region; fig. 1, rectangle). The numbered sites (49–64) outside the rectangle in figure 1 represent localities from which we collected comparative material elsewhere in the range of *Cnemidophorus tigris* sensu lato.

The region of contact between *C. t. punctilinealis* and *C. t. marmoratus* is in the northwestern part of the Animas Valley, east of the Peloncillo Mountains (fig. 3). This is the region where all contacts between these taxa were known at the beginning of our study. The 48 collecting sites within the contact region were primarily in desert-grassland or creosote desertscrub habitats at latitudes south of Redrock, Grant County, New Mexico, and longitudes between San Simon, Cochise County, Arizona (to the west) and Lordsburg, Hidalgo County, New Mexico (to the east; fig. 3). In figure 3, Animas Valley is to the east of the Peloncillo Mountains, which are oriented north–northwest to south–southeast to the west of Road Forks. The box indicating a town on the west (SSW of site 20) is San Simon, above the label for the riverbed of the same name. In the inset in figure 3, "grassland" refers to areas with essentially no bushes (or very widely scattered ones; but often with some *Yucca*), "playa" refers to alkali flats, and "other" refers primarily to farms but also to fallow fields and slopes with juniper woodland. Distribution of the major vegetative communities, mapped with different symbols and described in appendix 1, is based on our own surveys, conducted mostly in 1990. We personally observed the areas where vegetative communities are shown to abut each other abruptly (e.g., N of site 16 or SE of site 10), or where they overlap (e.g., site 15). Blank areas in figure 3 (e.g., to the east of the label reading "Road Forks") represent the limits to our determinations (e.g., in the large white area SW of site 29 we did not determine precisely where the creosote desertscrub, grass, and mesquite-dominated communities merged, so we left it blank).

Within the contact region (fig. 3) there are three separate hybrid zones where *C. t. punctilinealis* and *C. t. marmoratus* have been known to interbreed since before 1960 (Zweifel, 1962). We refer to these as the northern (fig. 4), central (fig. 5), and southern (fig. 5) hybrid zones. Collecting sites for each hybrid zone include a more-or-less straight transect across the zone of gene exchange, as well as associated sites relevant to understanding the transect. The hybrid zones and transects are illustrated in figures 4 and 5, the collecting sites relevant to each are specified in table 2, and the full locality data, habitat descriptions, and list of specimens examined for each site are detailed in appendix 1. Photographs of habitats within the contact region are presented in figures 6–22, and photography sites are documented in tables 2 and 3. The locality data and list of specimens examined from sites outside the contact region are presented in appendix 2.

Recently, Charles W. Painter and collaborators (personal commun.) have obtained evidence of a possible new contact, but involving only a few lizards with some *marmoratus*-like characters in the vicinity of the eastern end of Antelope Pass, the next significant pass through the Peloncillo Mountains off the bottom of figure 3. This area perhaps
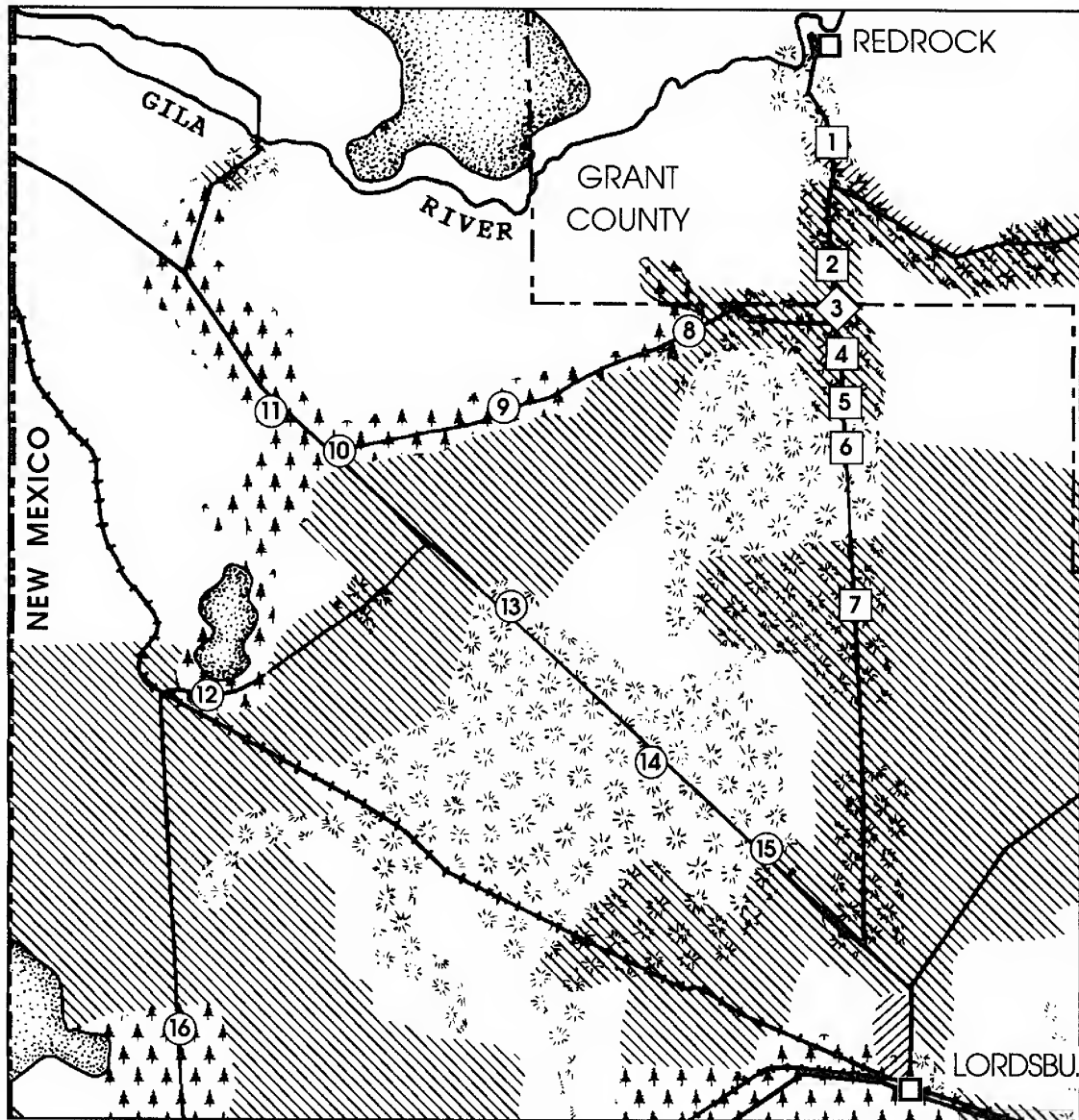
Fig. 4.   The northern hybrid zone, with collecting sites numbered (enlarged from fig. 3). Transect sites are numbers within squares (1–7), but the midpoint (50:50 point of gene exchange) is within a diamond (site 3). Associated sites are numbers in circles (table 2; appendix 1).

TABLE 2
Collecting Sites Within the Contact Region[a]: Three Transects and Associated Sites

Sites[b]	Coordinates[c]	Habitat	Figure[d]
	Northern Transect		
1	32°39′32.3″N, 108°44′24.8″W	Riparian thornscrub	6A
2	32°36′53.8″N, 108°44′15.2″W	Riparian scrub	6B
3	**32°36′10.0″N, 108°44′17.8″W**	**Riparian scrub**	**7A**
4	32°35′19.8″N, 108°44′5.2″W	Mesquite grassland	7B
5	32°34′26.2″N, 108°43′57.6″W	Mesquite grassland	8A
6	32°33′36.4″N, 108°43′56.9″W	Mesquite grassland	8B
7	32°30′33.1″N, 108°43′44.4″W	Mesquite grassland	9A
	Northern Associated Sites		
8	32°35′26.6″N, 108°47′47.9″W	Creosote desertscrub	9B
9	32°34′4.5″N, 108°51′53.8″W	Creosote desertscrub	—
10	32°33′16.9″N, 108°55′38.0″W	Creosote desertscrub	10A
11	32°34′27.7″N, 108°57′10.8″W	Creosote desertscrub	—
12	32°28′40.5″N, 108°58′50.6″W	Creosote desertscrub	—
13	32°30′11.8″N, 108°51′42.4″W	Mesquite grassland	—
14	32°27′43.9″N, 108°48′36.7″W	Mesquite grassland	11B
15	32°25′51.3″N, 108°46′10.0″W	Mesquite grassland	—
	Central Transect		
20	32°18′8.2″N, 109°10′32.7″W	Creosote desertscrub	—
21	32°13′32.5″N, 109°3′30.3″W	Creosote desertscrub	—
22	32°13′18.4″N, 109°0′18.2″W	Creosote desertscrub	—
23	32°13′19.1″N, 108°59′11.1″W	Creosote desertscrub	—
24	32°13′24.9″N, 108°58′41.3″W	Creosote desertscrub	—
25	32°13′24.4″N, 108°58′8.0″W	Creosote desertscrub	—
26	**32°13′24.6″N, 108°57′51.8″W**	**Creosote desertscrub**	**—**
27	32°13′26.3″N, 108°57′38.9″W	Creosote desertscrub	—
28	32°13′29.1″N, 108°57′12.2″W	Creosote desertscrub	12A
30	32°12′30.7″N, 108°56′29.1″W	Mesquite/tarbush	—
29	32°18′44.1″N, 108°48′46.1″W	Creosote desertscrub	—
	Central Associated Sites		
16	32°22′9.4″N, 108°58′57.5″W	Creosote desertscrub	16A
17	32°18′41.4″N, 108°58′30.0″W	Creosote desertscrub	—
18	32°18′6.5″N, 108°58′41.7″W	Creosote desertscrub	—
19	32°13′32.5″N, 109°3′30.3″W	Creosote desertscrub	—
31	32°10′44.0″N, 108°56′31.4″W	Creosote desertscrub	—
	Southern Transect		
36	32°7′9.0″N, 109°10′37.3″W	Creosote/mesquite	—
37	—	Creosote/mesquite	—
38	32°7′5.7″N, 109°1′30.5″W	Creosote desertscrub	—
39	32°4′54.8″N, 108°59′16.8″W	Creosote desertscrub	22B
40	32°6′18.3″N, 108°57′51.3″W	Creosote/tarbush	18A
42	**32°5′35.4″N, 108°56′50.8″W**	**Creosote desertscrub**	**18B**
44	—	Mesquite/creosote	—
46	—	Mesquite/snakeweed	—
48	—	Creosote/tarbush	—
	Southern Associated Sites		
32	32°9′2.0″N, 108°56′28.2″W	Creosote desertscrub	—
33	32°7′24.3″N, 108°55′46.7″W	Creosote desertscrub	20A
34	32°3′11.4″N, 108°56′21.3″W	Creosote desertscrub	—
35	—	Mesquite/graythorn	—
41	32°6′59.4″N, 108°56′29.6″W	Creosote desertscrub	19A
43	32°4′14.1″N, 108°56′39.9″W	Creosote/tarbush	—
45	—	Creosote/mesquite	—
47	—	Creosote desertscrub	—

should be considered in studies in the distant future (see "Hybridization With the Unisexual *C. tesselatus*" below). In addition, we have recently found evidence for another hybrid zone within the contact region, along the railroad tracks northwest of Lordsburg (see below, "Comparisons With the Past and Predictions for the Future").

HABITATS AND DISTRIBUTION

The inset in figure 3 identifies the habitats of greatest significance to *Cnemidophorus tigris* in the contact region. *Cnemidophorus t. punctilinealis* and *C. t. marmoratus* prefer the same habitats in the region (creosote and mesquite), and they also avoid the same habitats (grassland and playa). Therefore, hybridization in this case involves closely related taxa (see below, "Taxonomic Status of *C. t. marmoratus*") with similar ecological preferences and similar physiological characteristics (Dohm et al., 1998).

The preferred habitats are those referred to as "creosote" and "mesquite" in figure 3, between 1150 and 1400 m elevation. By "creosote" we mean desertscrub habitats where creosote bush (*Larrea divaricata*) is the dominant plant (for example, figs. 12, top and 17, bottom). At some sites (for example, fig. 21, bottom), tarbush (*Flourensia cernua*) also abounds, as may *Acacia* and mesquite (*Prosopis*). Soils usually are sandy, with gravel. These habitats are referred to by Brown (1982c) as "Chihuahuan desertscrub" and by Lowe (1964 and personal commun.) as "creosote desertscrub." By "mesquite" in figure 3 we mean habitats where *Prosopis* is the dominant plant (for example, figs. 8, bottom and 11, bottom). Soils at these sites often are sandy and mounded in hummocks beneath the bushes. In both of these habitats preferred by western whiptails there is open ground between most bushes, and the lizards

TABLE 3
Geographic Coordinates for Habitat Photographs Not Taken at Collecting Sites

Figure[a]	Coordinates[b]	View
9B	32°35′28.8″N, 108°47′38.2″W	NW
10B	32°32′59.9″N, 108°55′16.1″W	NNW
11A	32°31′27.8″N, 108°53′20.4″W	N
13A	32°14′14.2″N, 108°57′11.3″W	W
13B	32°14′14.2″N, 108°57′11.3″W	NE
14A	32°16′45.9″N, 108°52′46.6″W	SW
14B	32°16′45.9″N, 108°52′46.6″W	NE
15A	32°19′04.6″N, 108°48′53.0″W	SW
15B	32°19′04.6″N, 108°48′53.0″W	NE
16A	32°22′30.9″N, 108°58′55.3″W	S
16B	32°22′30.9″N, 108°58′55.3″W	W
17A	32°22′30.9″N, 108°58′55.3″W	N
17B	32°22′30.9″N, 108°58′55.3″W	NE
18A	32°06′08.1″N, 108°57′30.1″W	NW
18B	32°06′08.1″N, 108°57′30.1″W	SE
19A	32°06′57.2″N, 108°56′34.0″W	W
19B	32°06′57.2″N, 108°56′34.0″W	SE
20A	32°07′24.2″N, 108°56′17.4″W	E
20B	32°05′38.3″N, 108°58′06.3″W	SW
21A	32°05′22.4″N, 108°58′21.6″W	SW
21B	32°05′07.9″N, 108°58′48.5″W	W
22A	32°05′07.9″N, 108°58′48.5″W	NE
22B	32°04′25.0″N, 108°59′30.6″W	NE

[a] A refers to the top photograph in the figure, B to the bottom photograph.

[b] Coordinates were estimated using the Global Positioning System (WGS 84) and taking the mean of 10 readings per site with a Sony Pyxis receiver.

can dash from bush to bush with few obstacles. In places with considerable grass between the mesquite bushes it is appropriate to refer to this habitat as "semidesert grassland" (Brown, 1982b) or as "mesquite grassland" (Lowe, 1964 and personal commun.). At sandy localities with hummocks and little or no grass, "Chihuahuan Desertscrub" is as appropriate as "disclimax semidesert grassland" (Brown, 1982c).

←

[a] Figure 3 is a map of the contact region showing collecting sites; figures 4 and 5 are maps of the three transects. Sites 3, 26, and 42 are the 50:50 points of gene exchange (midpoints) of their respective transects.

[b] Appendix 1 presents complete locality data, description of habitats, and list of specimens examined.

[c] Geographic coordinates were estimated using the Global Positioning System (WGS 84) and taking the mean of 10 readings per site with a Sony Pyxis receiver.

[d] A refers to the top photograph in the figure, B to the bottom photograph.
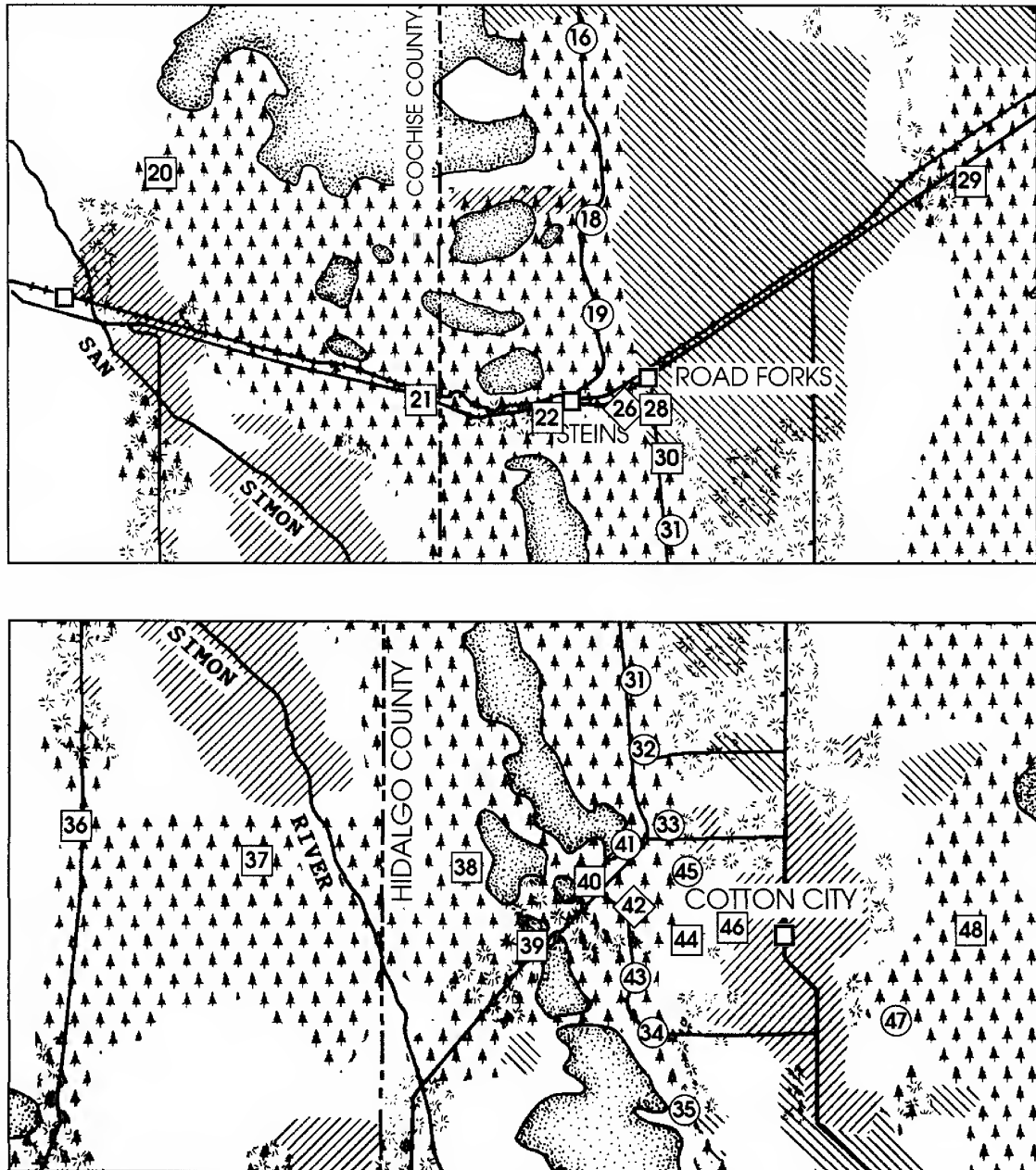
Fig. 5. **Top**. The central hybrid zone (enlarged from fig. 3), with collecting sites numbered as in figure 4. Site 26 is the midpoint (50:50 point of gene exchange) in the transect (sites 20–30; table 2; appendix 1). Sites 23–25 are between sites 22 and 26, while site 27 is between sites 26 and 28. Note that site 30 comes before site 29 (reading left to right) in the transect from west to east. **Bottom**. The southern hybrid zone (enlarged from fig. 3), with collecting sites numbered as in figure 4. Site 42 is the midpoint (50:50 point of gene exchange) in the transect (sites 36–40, 42, 44, 46, and 48; table 2; appendix 1).

Habitats (fig. 3) that are avoided by *C. tigris* are the higher elevation foothills and mountains with juniper woodland and other tree communities, ''grassland/playa'' (alkali flats, for example, fig. 14), and ''other'' habitats (mostly farms and fallow fields). By ''grassland'' in figure 3 we mean areas where grasses abound and mesquite or other bushes and shrubs are very few or widely scattered, if present (for example, figs. 11, top and 16, bottom), although *Yucca elata* usually is present (often widely scattered). This may be referred to as ''desert-grassland'' or ''semi-desert grassland'' (Lowe, 1964; Brown, 1982b).

Although *C. tigris* avoids the habitats just mentioned, other whiptail lizards are found in those areas (table 1). *Cnemidophorus exsanguis* and *C. sonorae* are triploid parthenogens that occur in foothill and montane woodlands. *Cnemidophorus uniparens* is a triploid parthenogen that occurs in all of the lower elevation habitats (creosote desert-scrub, mesquite, and grassland). *Cnemidophorus neomexicanus* is a diploid parthenogen that occurs mostly in creosote/mesquite-grassland ecotones. *Cnemidophorus inornatus* is a bisexual species that occurs in the grasslands northwest of Lordsburg. Finally, *Cnemidophorus tesselatus* type F (= *C. dixoni*) is a diploid parthenogen that occurs in creosote desertscrub a few kilometers off the bottom of the map (fig. 3), in Antelope Pass, Peloncillo Mountains, New Mexico, west of Animas (Zweifel, 1965; Degenhardt et al., 1996). Remarkably, *C. tigris* is known to hybridize rarely with four of these six other species, and it is one of the bisexual ancestors for two of the unisexual taxa (*C. neomexicanus* and *C. tesselatus*), which are of hybrid origin. These additional phenomena of hybridization among *C. tigris* and other species are discussed below (see ''Interspecific Hybridization'').

After presenting the genetic and morphological data, we revise our earlier suggestion that one of these hybrid zones may be moving in space and time. Present analyses do not support that hypothesis.

BIOCHEMICAL GENETICS

The database on molecular genetics of *C. tigris* from the contact region includes evidence on the 12S ribosomal mitochondrial DNA locus and on 36 protein-encoding nuclear loci for 607 lizards from 48 sites. Such evidence also was obtained for 47 specimens of *C. t. punctilinealis* and *C. t. marmoratus* from sites outside the contact region. For comparative purposes, data on most of the same protein loci were obtained for 29 specimens of the subspecies *C. t. septentrionalis*, which occurs immediately to the north, and five specimens of *C. t. aethiops*, which occurs immediately to the south of the range of *C. t. punctilinealis* (fig. 1).

METHODS

TISSUE SAMPLING

The heart, liver, kidney, stomach, small intestine, and samples of skeletal muscle and blood were collected from freshly killed lizards. The pancreas, visible parasites, and food remains were removed, and plasma was separated from blood cells by centrifugation prior to freezing. Both blood fractions and other tissues were frozen in liquid nitrogen. Until used for experimental work, tissues

TABLE 4
Sequences of ASO Probes Used[a]

Sample[b]	Sequence	T_m[c], °C
MAR	CCA ATA GTC CAC CAA CTA	52
MAR2	CCA ATA GTC CAC CAA	44
PUN	CTA ATA GTT TCT CAA CTA	46
PUN Tucson	ATA GTT CTT CAA CTA	38
INO	CCA ACA GTC TAC CAA CTA	52

[a] Allele-specific oligonucleotide (ASO) probe designs are based on positions 202–219 from the 5′-phosphate end of sequences of PCR product of the 12S ribosomal mtDNA gene of *Cnemidophorus* taxa (Reeder et al., unpubl. data; see Dessauer et al., 1996b).

[b] MAR = *Cnemidophorus tigris marmoratus* (MAR2 is simply shorter); PUN = *C. t. punctilinealis* from the contact region; PUN Tucson = *C. t. punctilinealis* from sites 49 and 56; INO = *C. inornatus*.

[c] Estimate of the melting temperature of the ASO: $T_m = 4(\#G + \#C) + 2(\#A + \#T)$.

TABLE 5
Presumptive Structural Gene Loci Examined in *Cnemidophorus tigris*

Locus[a]	Ec. no.	Abbre- viation	Sub- unit no.[b]	Tissue distr.[c]	Buffer[d]
Oxidoreductases					
Alcohol dehydrogenase	1.1.1.1	ADH	2	**L**>K	TM 8
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	1	**M**>L>H	PC 6
L-iditol dehydrogenase	1.1.1.14	IDDH	4	**K**>D>L	PC 6, TM 8
L-lactate dehydrogenase	1.1.1.27	LDH1	4	H>**K**>D,L	PC 6, TM 8
		LDH2	4	M,L>D>**K**	PC 6, TM 8
Malate dehydrogenase	1.1.1.37	sMDH	2	H>**M**>L,K	PC 6
		mMDH	2	H>**M**>L,K	PC 6
Malate enzyme	1.1.1.40	sMDHP	4	**K**>M>L	TM 8 NADP
Isocitrate dehydrogenase	1.1.1.42	sIDH	2	**L**>K,H	TM 8 NADP
		mIDH	2	**M**,H>K,L	TM 8 NADP
Superoxide dismutase	1.15.1.1	sSOD	2	**L**>K,D>H	TB 8.6 EDTA
		mSOD	2	**L**,D>K,H	TB 8.6 EDTA
Diaphorase	1.6.4.3	DDH	—	**L**>K	TB 8.6 EDTA
Transferases					
Aspartate aminotransferase	2.6.1.1	sAAT	2	H>L>**M**,K	PC 6
		mAAT	2	**M**,H,L,K	PC 6
Creatine kinase	2.7.3.2	CK1	2	**K**,S,L,M	PC 6
		CK2	—	**H**>M	PC 6
Adenylate kinase	2.7.4.3	AK	—	**M**>K,H,L	PC 6
Hydrolases					
Esterase 1	3.1.–.–	EST1[e]	1	K>L>**M**,D	PC 6
Esterase 2	3.1.–.–	EST2[f]	1	L>**M**>K	PC 6
Esterase D	3.1.1.–	ESTD[g]	2	**M**,L>H,K	PC 6
Alkaline phosphatase	3.1.3.1	ALP[h]	—	**L**	TC 8
Peptidases	3.4.–.–	PEPA[i]	2	K>**M**,L,H	PC 6
		PEPB[j]	2	L>K>**M**,H	PC 6
		PEPE[j]	—	**M**,K,R	PC 6
	3.4.13.9	PEPD[k]	2	**R**>K,L,H	PC 6
Adenosine deaminase	3.5.4.4	ADA	1	R>**L**,K	PC 6
Lyases					
Aconitate hydratase	4.2.1.3	sACOH	1	**L**>K	PC 8
		mACOH	—	H,K>**L**	PC 8
Isomerases					
Mannose-6-phosphate isomerase	5.3.1.8	MPI	1	**L**>K,R	PC 6
Glucose-6-phosphate isomerase	5.3.1.9	GPI	2	**M**>H>K,L	PC 6
Phosphoglucomutase	5.4.2.2	PGM1	1	**M**≫L>R,H	PC 6
		PGM2	1	**M**,L>R,H	PC 6
		PGM3	1	**L**>K	PC 6
Nonenzymic Blood Proteins					
Transferrin		TF	1	**P**	V 8.6; B 8.6
Hemoglobin		HB	—	**R**	PC 6

[a] Methods are basically as described by Murphy et al. (1996); s = cytosolic enzyme; m = mitochondrial enzyme. For multilocus systems, loci are numbered in order of decreasing anodal migration of their polypeptide products.

[b] Number of subunits in the enzyme is based on isozyme patterns observed in heterozygous individuals.

[c] Tissue distribution: D = duodenum; H = heart; K = kidney; L = liver; M = skeletal muscle; R = red cells; P = plasma; S = stomach; tissues used most frequently in analyses are in bold type.

[d] Buffer components, pH, and additives: B = boric acid; C = citric acid; M = maleic acid; P = disodium hydrogen phosphate; T = tris; V = barbituric acid (veronal).

[e] Substrate 4-methylumbelliferyl butyrate.

were maintained in ultracold freezers at the Louisiana State University Medical Center, New Orleans. Methods of tissue storage and curation have been described elsewhere (Dessauer et al., 1996a).

MITOCHONDRIAL DNA TYPING

DNA was isolated from red blood cells, skeletal muscle, or liver using standard phenol-chloroform extractions. DNA concentrations and quality were confirmed by UV absorption of aqueous solutions and ethidium bromide fluorescence of polymerase chain reaction (PCR) products in agarose gels (Maniatis et al., 1982).

Fragments of approximately 368 base pairs of the mitochondrial 12S ribosomal genes (12S mtDNA) of *C. t. marmoratus* and *C. t. punctilinealis* from the contact region and *C. t. punctilinealis* from west of there (sites 49 and 56, referred to below as the "Tucson area") were amplified by PCR using universal primers H1557 and L1091 (Knight and Mindell, 1993). Tod W. Reeder sequenced and aligned the PCR products.

Mitochondrial DNA (mtDNA) typing was carried out using dot-blot technology, a rapid and efficient method for screening DNA haplotypes (Dessauer et al., 1996b). The dot-blot method utilizes allele-specific oligonucleotides (ASOs), that is, short, single-stranded nucleotide sequences that incorporate features that distinguish the sequences of the different mtDNAs of *C. tigris*. As a probe, an ASO hybridizes to complementary sequences on strands of DNA (Saiki et al., 1986).

Fragments of the mitochondrial 12S ribosomal gene (12S mtDNA) for each lizard were amplified by PCR and then subjected to dot-blot analysis. The allele-specific oligonucleotides used in these tests (table 4) were designed from nucleotide differences within positions 202 through 219 of sequences of the 12S ribosomal mtDNAs of *C. t. marmoratus* (ASO-MAR), *C. t. punctilinealis* (ASO-PUN), and *C. t. punctilinealis* from the Tucson area (ASO-PUN Tucson). Details of the dot-blot method are described in Saiki et al. (1986) and Dessauer et al. (1996b).

PROTEIN ANALYSES

For electrophoretic analysis of proteins, tissues were homogenized in two to three volumes of buffer (0.25 M sucrose, 20 mg dithiothreitol, 100 mg NAD, and 10 mg NADP per liter) and centrifuged at 10,000 × *g* to separate the supernatant solution of soluble proteins from cell debris. Aliquots of homogenates, hemolysates, or blood plasma were added to slots in starch gels and subjected to vertical gel electrophoresis (Smithies, 1959).

Enzymes and nonenzymic proteins were localized on gel slices, closely following descriptions by Harris and Hopkinson (1976). Transferrins were identified by iron-59 binding and autoradiography (Giblett et al., 1959). Table 5 lists the 36 loci scored, their enzyme commission numbers, abbreviations (Murphy et al., 1996), subunit numbers, tissues that gave the best resolution for each protein, and buffers used in their analysis. For multilocus enzymes, loci are labeled numerically in order of decreasing anodal migration of their isozymes. Similarly, alleles at specific loci are labeled alphabetically in order of decreasing anodal migration of their allozymes.

Proteins determined by 14 additional loci were localized for many lizards, but were not scored routinely for every individual. These included EST3 and acid phosphatase scored in our earlier study (Dessauer and Cole, 1991). The probable presence of null alleles made EST3 impossible to score for all liz-

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[f] Substrate 4-methylumbelliferyl acetate.

[g] Substrate 4-methylumbelliferyl acetate or 4-methylumbelliferyl butyrate; inactive with alpha-naphthyl esters.

[h] Substrate alpha-naphthyl acid phosphate; liver extracted with *n*-butanol.

[i] Substrate phenylalanyl.leucine.

[j] Substrate leucyl.glycyl.glycine.

[k] Substrate phenylalanyl.proline.

Fig. 6.   Habitats at collecting sites in the northern transect, Animas Valley, north of Lordsburg (table 2; figs. 3, 4; appendix 1), 22 August 1990. **Top**. Site 1, looking E from NM Hwy 464; riparian thorn-scrub. **Bottom**. Site 2, looking W from NM Hwy 464.

Fig. 7. Habitats at collecting sites in the northern transect, Animas Valley, north of Lordsburg (table 2; figs. 3, 4; appendix 1), 22 August 1990. **Top**. Site 3, midpoint of the northern hybrid zone, looking E from NM Hwy 464. **Bottom**. Site 4, looking NW from NM Hwy 464; mesquite grassland.

Fig. 8.   Habitats at collecting sites in the northern transect, Animas Valley, north of Lordsburg (table 2; figs. 3, 4; appendix 1), 22 August 1990. **Top**. Site 5, looking W from NM Hwy 464; mesquite grassland. **Bottom**. Site 6, looking W from NM Hwy 464.

Fig. 9.   Habitats at collecting sites in the northern transect and near an associated site, Animas Valley, north of Lordsburg (tables 2, 3; figs. 3, 4; appendix 1), 22 August 1990. **Top**. Site 7, looking W from NM Hwy 464. **Bottom**. Abrupt ecotone of mesquite grassland and creosote desertscrub (in near background), 5.8 km (by road) W of site 3, looking NW. Site 8 is 0.3 km off the left side of the photo.

Fig. 10.   Habitats near the northern transect (tables 2, 3; figs. 3, 4; appendix 1), Animas Valley, NW Lordsburg along NM Hwy 70, 2 September 1990. **Top**. Site 10, looking NNW; creosote desertscrub. **Bottom**. Grassland and abrupt ecotone with creosote desertscrub (in near background), 32.1 km (by road) NW of Lordsburg, looking NNW; site 10 is 0.8 km to the NW.

Fig. 11. Habitats near the northern transect (tables 2, 3; figs. 3, 4; appendix 1), Animas Valley, NW of Lordsburg along NM Hwy 70, 2 September 1990. **Top**. Grassland about halfway between sites 10 and 13, at 27.9 km (by road) NW Lordsburg, looking N. **Bottom**. Site 14, looking WSW.

Fig. 12.   Habitats in the central transect (table 2; figs. 3, 5; appendix 1), between San Simon and Lordsburg, 22 August 1990. **Top**. Site 28, looking W at Steins Pass from NM Hwy 80 along the gas pipeline road; creosote desertscrub. **Bottom**. Looking E from site 28; grassland and alkali flats in near background.

Fig. 13.   Habitats near the central transect (tables 2, 3; figs. 3, 5; appendix 1), between San Simon and Lordsburg, 22 August 1990. **Top**. Looking W at Steins Pass from the intersection of NM Hwy 80 and Hwy I-10, Road Forks; grassland, with creosote desertscrub visible in the pass. **Bottom**. Looking NE at grassland and alkali flats in Animas Valley, from the same place as the top photograph.

Fig. 14.   Habitats near the central transect (tables 2, 3; figs. 3, 5; appendix 1), between Road Forks and Lordsburg, Animas Valley, 22 August 1990. **Top**. Intersection of NM Hwy 338 and Hwy I-10, about halfway between Road Forks and site 29, looking SW at alkali flats. **Bottom**. Looking NE at alkali flats, with creosote desertscrub in near background, from the same place as the top photograph.

Fig. 15. Habitats near the central transect (tables 2, 3; figs. 3, 5; appendix 1), between Road Forks and Lordsburg, Animas Valley, 22 August 1990. **Top**. Looking SW along Hwy I-10, just N of site 29. **Bottom**. Looking NE along Hwy I-10 from the same place as the top photograph (note creosote bushes on right).

Fig. 16.   Habitats N of Steins, near the central transect (tables 2, 3; figs. 3, 5; appendix 1), 23 August 1990. **Top**. Looking S behind the allelemobile at abrupt ecotone between grassland and creosote desertscrub, 0.8 km N of site 16. **Bottom**. Looking W across grassland, with creosote ecotone on left (in near background), from the same place as the top photograph.

Fig. 17. Habitats N of Steins, near the central transect (tables 2, 3; figs. 3, 5; appendix 1), 23 August 1990. **Top**. Looking N (from low hill behind allelemobile in fig. 16, top) across the grassland that separates site 16 (*marmoratus*) and site 12 (largely *punctilinealis*; figs. 3, 49). **Bottom**. Looking NE at grassland and alkali flats, from same place as the top photograph.

Fig. 18.   Habitats in the southern transect, W of Cotton City (tables 2, 3; figs. 3, 5; appendix 1), 22 August 1990. **Top**. Looking NW from NM Hwy 80, at 16.0 km (by road) S of Road Forks; site 40 is 0.7 km to the NW. **Bottom**. Looking SE (from the same place as the top photograph); site 42 is 1.5 km to the SE; Table Top Mountain (site 47) is the sloping mesa across Animas Valley (in far background, left of center).
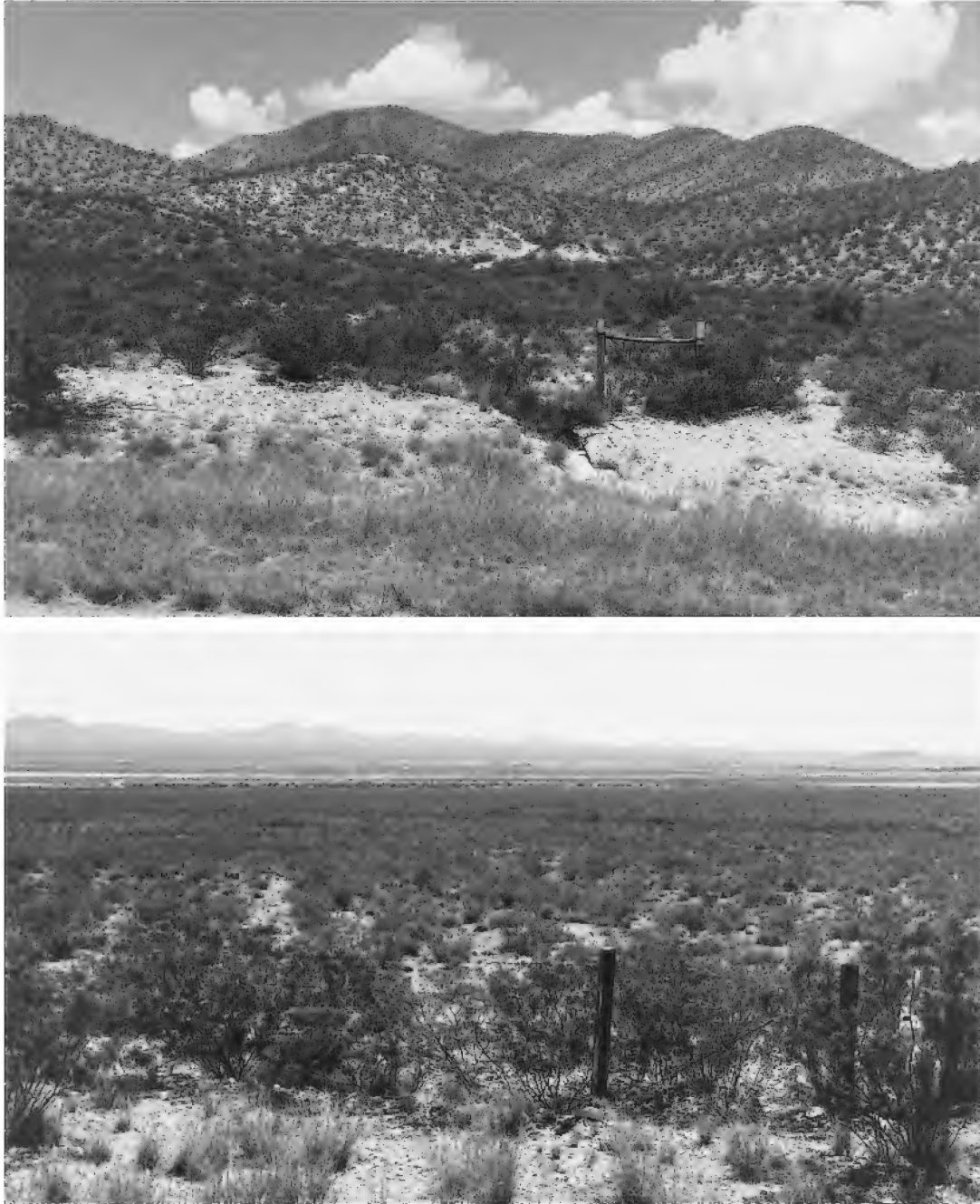
Fig. 19. Habitats near the southern transect, W of Cotton City (tables 2, 3; figs. 3, 5; appendix 1), 22 August 1990. **Top**. Site 41, Crystal Mine, looking W from NM Hwy 80, at 13.7 km (by road) S of Road Forks. **Bottom**. Looking SE from the same place as the top photograph; Table Top Mountain (site 47) is the low butte in the distant right.

Fig. 20.   Habitats near the southern transect, along NM Hwy 80 S of Road Forks (tables 2, 3; figs. 3, 5; appendix 1), 22 August 1990. **Top**. Looking E from 12.8 km (by road) S of Road Forks; site 33 is 0.8 km to the E (on left). **Bottom**. Looking SW at Granite Gap in the Peloncillo Mountains from 17.7 km (by road) S of Road Forks.
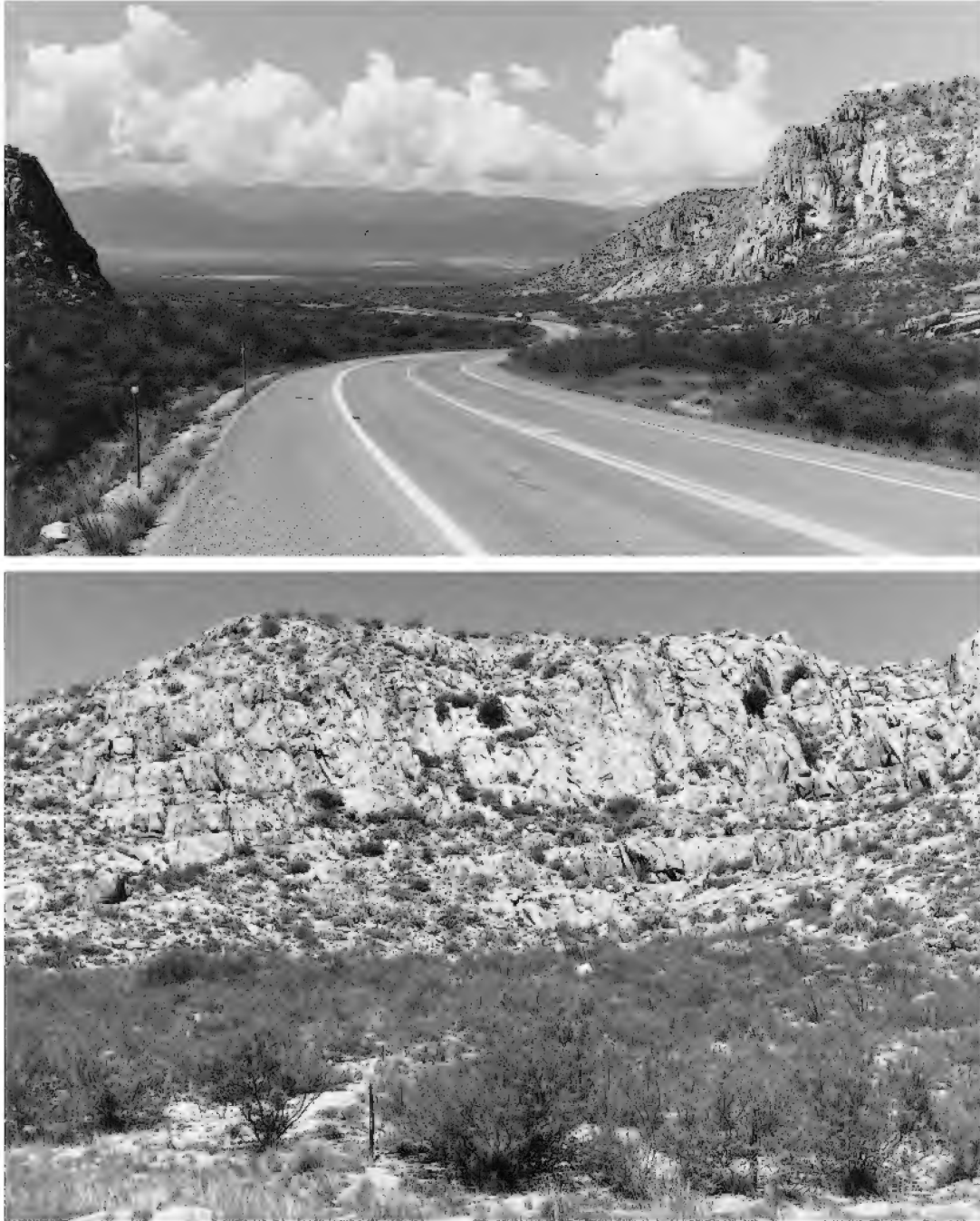
Fig. 21.   Habitats near the southern transect, along NM Hwy 80 S of Road Forks (tables 2, 3; figs. 3, 5; appendix 1), 22 August 1990. **Top**. Looking SW through Granite Gap from 18.2 km (by road) S of Road Forks. **Bottom**. Looking W in Granite Gap from 19.0 km (by road) S of Road Forks.

Fig. 22.   Habitats S of the southern transect, along NM Hwy 80 S of Road Forks (tables 2, 3; figs. 3, 5; appendix 1), 22 August 1990. **Top**. Looking NE in Granite Gap, from the same place as figure 21, bottom. **Bottom**. Looking NE at Granite Gap from 20.7 km (by road) S of Road Forks; site 39 is 1.2 km to the NE (on left).

ards. Acid phosphatase phenotypes were not consistently reproducible, probably explaining why presumed allele frequencies at that locus were found at times to deviate from Hardy-Weinberg equilibrium in our earlier study (probably an artifact). Polymorphic prealbumin was excluded because albumin migrated in the same area of the gel as prealbumin and often masked its phenotypes. Other activities localized but not scored consistently were mitochondrial type malic enzyme, glucose-6-phosphate dehydrogenase, phosphogluconate dehydrogenase, glyceraldehyde phosphate dehydrogenase, NADH-linked diaphorase, pyruvate kinase, beta-galactosidase, PEPC, aldolase, albumin, and myoglobin.

IDENTIFICATION OF PROTEIN LOCI

Patterns of variation were scored for 36 structural gene loci. Identification of activities determined by these nuclear loci was based on substrate and cofactor requirements, as well as on tissue and organelle distributions. Identifications were easy for most oxidoreductases, transferases, and isomerases that have relatively specific substrate and cofactor requirements, but identifications were complicated for many hydrolases. For example, many esterases and peptidases recognize a wide variety of compounds as substrates, often with overlapping affinities. Esterase patterns commonly exhibit 20–30 isozymes when developed with alpha-naphthyl or 4-methyl-umbelliferyl esters. We were able to distinguish conclusively four esterase loci based on differential activities with three substrates. Alpha-naphthyl-acetate was utilized as a substrate for all esterases except ESTD. All four esterase loci utilized 4-methyl-umbelliferyl substrates but with different efficiencies. EST1 and EST3 were most active with 4-methyl-umbelliferyl-butyrate and EST2 with 4-methyl-umbelliferyl-acetate (fig. 25). Many peptidases, including PEPA, PEPB, and PEPE, utilized phenylalanyl.leucine as a substrate (Rapley et al., 1971). To distinguish among these peptidases, more specific substrates were selected: valyl.leucine for PEPA, leucyl.glycyl.glycine for PEPB and E, and phenylalanyl.proline for

PEPD (Harris and Hopkinson, 1976; Dessauer and Braun, 1988).

The number and relative activity of isozymes that make up the electrophoretic phenotype generally conform closely to values expected for a random association of the polypeptide subunits (Neaves and Gerald, 1969; Dessauer and Cole, 1984). Thus, for heterozygous individuals of diploid *C. t. punctilinealis* and *C. t. marmoratus*, phenotypes of monomeric proteins have two-banded patterns with the bands approximating a 1:1 ratio of activities (for example, EST2 [fig. 25]; sACOH, PGM1, PGM2, PGM3 [fig. 26]; and TF [fig. 27]). Heterozygotes of dimeric enzymes have three-banded patterns with the bands approximating a 1:2:1 ratio of activities (for example, MDH and AAT [fig. 33; see "Uncommon Alleles" below]; IDH, ESTD [fig. 51; see "Comparisons with Additional Subspecies of *C. tigris*" below]; PEPB; and PEPD and GPI [fig. 29]). Heterozygotes of tetrameric enzymes have five-banded patterns approximating a 1:4:6:4:1 ratio of activities (for example, IDDH [fig. 27] and LDH [fig. 52]; see "Hybridization With the Unisexual *C. neomexicanus*" below).

The distribution of isozymes in different organelles or tissues affected the identification and scoring of activities. For example, differences in isozyme patterns of cytosolic and mitochondrial forms of a protein helped distinguish patterns of sMDH from mMDH, sIDH from mIDH, and sAAT from mAAT. Choice of tissue often simplified analyses. PGM1 and PGM2 were readily resolved in muscle and PGM2 and PGM3 in liver (fig. 26). Liver was almost exclusively the site of ADH activity and kidney of IDDH activity (use of homogenates of single tissues rather than tissue mixtures avoided diluting concentrations of these enzymes). Subunits of LDH1 and LDH2 associate so that banding patterns for a tissue such as liver, in which both loci are active, include isozymes that are hybrids of two loci. Use of kidney or heart homogenates in which LDH2 is largely inactive simplified the scoring of LDH1, and use of muscle homogenates in which LDH1 activity is low simplified scoring of LDH2 (fig. 52; see "Hybridization With the Unisexual *C. neomexicanus*" below). Use of

muscle rather than liver homogenates facilitated scoring of EST2, as the extremely high esterase activities in liver masked EST2 phenotypes.

DATA ANALYSES

Molecular databases were analyzed for F statistics, heterozygosities, and Nei's genetic distance with the BIOSYS-1 program, version 1.7 (Swofford and Selander, 1981). GENEPOP, version 1.2 (Raymond and Rousset, 1995), and the GDA-Program-Version-d11 (Lewis and Zaykin, 1998) were both used to test loci for Hardy-Weinberg equilibrium, linkage equilibrium, and F statistics. The DFIT group of genetic programs was used for tracking patterns of variation of nuclear genes (protein alleles) with cytoplasmic genes (12S ribosomal mtDNA haplotypes). These programs, developed by Asmussen et al. (1987), also were used to evaluate cytonuclear disequilibria for possible explanations of mating patterns in the hybrid zones.

RESULTS

Allele and genotype frequencies in mtDNA and the nuclear protein loci illustrate clinal changes along transects, provide databases for calculating indices of diversity, and provide insights on mating patterns and fitness. The indices include (1) the distribution of lizards with different combinations of *punctilinealis* and *marmoratus* alleles, (2) genetic distances, (3) heterozygosities and F statistics, and (4) Hardy-Weinberg, linkage, and cytonuclear disequilibria. These indices are used to infer mating patterns of lizards from individual sites, gene flow, and whether selection against hybrids is an important determinant of genetic structure in each hybrid zone.

DIVERSITY AT THE 12S RIBOSOMAL MTDNA LOCUS

Sequences of 368 nucleotides of the mtDNA 12S ribosomal gene of *C. t. punctilinealis* from the contact region and from the area around Tucson (sites 49 and 56) differed from each other and from the sequence of the mtDNA of *C. t. marmoratus*, as determined by Tod Reeder (Dessauer et al., 1996b). Se-

quences of *punctilinealis* from the two areas differed by approximately eight nucleotides (5 transitions, 1 transversion, and 2 deletions, or 2.2%). The sequence for *marmoratus* differed by approximately 17 of the 368 nucleotides from the sequence of *punctilinealis* of the contact region (4.6%). The number of nucleotide differences were nearly the same, but their types were different in comparing *marmoratus* with the two haplotypes of *punctilinealis* (mtDNA from the contact zone: 10 transitions, 3 transversions and 4 deletions, or 4.6% differences; from the Tucson area: 9 transitions, 4 transversions, and 5 deletions, or 4.9% differences).

Differences in the sequences of mtDNA allowed us to identify the subspecific source of the maternal complement of mtDNA for all 607 specimens of *C. tigris* from the contact region. The identifications were based on the specificity of base-pairing of synthetic oligonucleotide probes to fragments of single stranded 12S ribosomal mtDNA. The dot-blots of figure 23 illustrate the specificity of the probes. ASO-MAR paired with mtDNA of *C. t. marmoratus* from the Animas Valley and from distant areas to the east. ASO-PUN paired with mtDNA of *C. t. punctilinealis* from the San Simon Valley and from the northern area of the Animas Valley south of the Gila River. However, mtDNA of *punctilinealis* from sites 49 and 56 near Tucson failed to pair with ASO-PUN. A modified oligonucleotide probe (ASO-PUN Tucson) was required to identify specimens from the two westernmost sites.

The dot-blots of figures 23 and 24 illustrate determination of mtDNA haplotypes for individuals from 32 collecting sites. Tables 6–12 and figures 30–32 show the distribution of mtDNA haplotypes for lizards from the different collecting sites, and appendix 3 lists the mtDNA haplotype for each lizard from the contact region.

DIVERSITY AT THE 36 PROTEIN LOCI

Tables 6, 8, and 10 tabulate frequencies of genotypes for lizards collected at sites across the transects of three hybrid zones (northern, central, and southern). Tables 7, 9, and 11 give frequencies of genotypes for lizards collected at sites associated with the three tran-

TABLE 6

Frequencies of Genotypes at Collecting Sites Along the Northern Transect[a]

Locus[b]	Site[c]						
	1 (10)	2 (9)	**3 (20)**	4 (10)	5 (10)	6 (10)	7 (10)
Oxidoreductases							
ADH							
aa	10	9	**20**	10	10	10	10
G3PDH							
aa	10	9	**20**	10	10	10	10
IDDH							
aa	—	—	**1**	2	2	1	1
ab	—	1	**4**	3	6	4	6
bb	10	8	**15**	5	2	5	3
LDH1							
aa	10	9	**20**	10	10	10	10
LDH2							
bb	10	9	**20**	10	10	10	10
sMDH							
bb	10	9	**20**	10	10	10	10
mMDH							
bb	10	9	**20**	10	10	10	10
sMDHP							
aa	—	1	**2**	7	6	3	7
ab	—	3	**9**	2	3	6	3
bb	10	5	**9**	1	1	1	—
sIDH							
aa	—	—	**—**	—	—	1	—
ab	—	—	**2**	—	1	—	—
bb	10	9	**18**	10	9	9	10
mIDH							
aa	10	9	**20**	10	10	10	10
sSOD							
aa	10	9	**20**	10	10	10	10
mSOD							
aa	10	9	**20**	10	10	10	10
DDH							
aa	10	9	**20**	10	10	10	10
Transferases							
sAAT							
bb	10	9	**20**	10	10	10	10
mAAT							
bb	10	9	**20**	10	10	10	10
AK							
aa	10	9	**20**	10	10	10	10
CK1							
bb	10	9	**20**	10	10	10	10
CK2							
aa	10	9	**20**	10	10	10	10
Hydrolases							
ALP							
aa	8	9	**20**	**10**	**10**	**10**	**10**
EST1							
bb	9	9	**20**	**10**	9	9	9
bc	1	—	**—**	**—**	1	1	1

TABLE 6—(*Continued*)

Locus[b]	Site[c]						
	1 (10)	2 (9)	**3 (20)**	4 (10)	5 (10)	6 (10)	7 (10)
EST2							
aa	—	1	4	3	4	7	10
ab	1	5	10	7	6	3	—
bb	9	3	6	—	—	—	—
ESTD							
bb	10	9	**20**	10	10	10	10
PEPA							
bb	10	9	**19**	9	9	10	7
bc	—	—	**1**	1	1	—	3
PEPB							
bb	7	5	**8**	—	—	—	—
bc	3	4	**4**	2	1	1	—
cc	—	—	**7**	6	8	8	8
bd	—	—	**1**	—	—	—	—
cd	—	—	**—**	2	1	1	2
PEPD							
aa	4	1	**6**	—	—	—	—
ab	2	2	**5**	—	—	—	—
bb	1	—	**1**	—	—	—	—
ac	—	—	**3**	2	3	1	—
bc	3	3	**1**	4	—	—	—
cc	—	1	**2**	2	5	4	6
bd	—	—	**1**	—	—	—	—
cd	—	—	**—**	—	—	1	—
ee	—	—	**—**	—	1	—	—
ae	—	1	**—**	—	—	2	1
ce	—	1	**1**	2	1	2	3
PEPE							
aa	10	9	**18**	10	10	10	10
ADA							
bb	10	9	**20**	9	10	10	10
Lyases							
sACOH							
ab	—	—	**—**	2	—	—	—
bb	10	9	**20**	8	10	10	10
mACOH							
aa	10	9	**20**	10	10	10	10
Isomerases							
MPI							
ab	—	—	**—**	1	1	—	—
bb	10	9	**20**	9	9	10	10
GPI							
aa	—	—	**2**	4	1	1	1
ab	—	—	**1**	2	6	3	1
bb	—	1	**2**	—	2	4	6
ac	2	3	**4**	2	1	2	1
bc	2	4	**5**	1	—	—	—
cc	6	1	**6**	1	—	—	1
PGM1							
aa	10	9	**20**	10	10	10	10
PGM2							
dd	10	9	**20**	10	10	10	10

TABLE 6–(*Continued*)

Locus[b]	Site[c]						
	1 (10)	2 (9)	**3 (20)**	4 (10)	5 (10)	6 (10)	7 (10)
PGM3							
aa	9	7	**17**	8	10	10	10
ab	1	1	**3**	2	—	—	—
ac	—	1	**—**	—	—	—	—
Nonenzymic Blood Proteins							
TF							
aa	—	1	**6**	6	5	8	10
ab	1	2	**8**	4	4	2	—
bb	9	6	**5**	—	1	—	—
HB							
aa	10	9	**20**	10	10	10	10
Mitochondrial DNA							
12s mtDNA							
P	10	6	**10**	1	—	2	—
M	—	3	**10**	9	10	8	10

[a] The collecting sites and northern transect are defined in table 2 and mapped in figures 3 and 4. Site 3 is the 50:50 point of gene exchange (midpoint).

[b] Locus abbreviations are defined in table 5. Alleles for protein loci are designated in alphabetical sequence in order of decreasing anodal migration. The 12S mtDNA data pertain to the allele-specific oligonucleotides (table 4; figs. 23 and 24); P refers to the haplotype of *C. t. punctilinealis*; M refers to the haplotype of *C. t. marmoratus*.

[c] Total lizards studied for respective sites are listed in parentheses. Where the number of observations within columns do not total the number of lizards in the sample, data were missing for one or more individuals.

TABLE 7

Frequencies of Genotypes at Collecting Sites Associated with the Northern Transect[a]

Locus[b]	Site[c]							
	8 (2)	9 (10)	10 (10)	11 (10)	12 (10)	13 (10)	14 (88)	15 (3)
	Oxidoreductases							
ADH								
aa	2	10	10	10	10	10	88	2
G3PDH								
aa	2	10	10	10	10	10	87	3
ab	—	—	—	—	—	—	1	—
IDDH								
aa	—	—	—	—	—	4	10	—
ab	1	2	—	—	1	5	41	1
bb	1	8	10	10	9	1	37	1
LDH1								
aa	2	10	10	10	10	10	88	3
LDH2								
bb	2	10	10	10	10	10	88	3
sMDH								
bb	2	10	10	10	10	10	88	3
mMDH								
bb	2	10	10	10	10	10	88	3
sMDHP								
aa	—	—	2	—	1	5	80	2
ab	1	—	2	2	—	5	8	1
bb	1	10	6	8	9	—	—	—
sIDH								
ab	—	—	—	—	—	—	1	—
bb	2	10	10	10	10	10	86	3
mIDH								
aa	2	10	10	10	10	10	87	3
sSOD								
aa	2	10	10	10	10	10	88	3
mSOD								
aa	2	10	10	10	10	10	87	3
DDH								
aa	2	10	10	10	10	10	88	2
	Transferases							
sAAT								
bb	2	10	10	10	10	10	87	3
bc	—	—	—	—	—	—	1	—
mAAT								
ab	1	—	—	1	1	—	—	—
bb	1	10	8	8	8	10	87	3
bc	—	—	2	1	1	—	—	—
AK								
aa	2	10	10	10	10	10	88	3
CK1								
ab	—	—	—	—	—	1	1	—
bb	2	10	10	10	10	9	87	2
CK2								
aa	2	10	10	10	10	10	88	3

TABLE 7—(*Continued*)

Locus[b]	Site[c]							
	8 (2)	9 (10)	10 (10)	11 (10)	12 (10)	13 (10)	14 (88)	15 (3)
Hydrolases								
ALP								
aa	2	10	10	10	10	10	85	3
EST1								
ab	—	—	—	—	1	—	—	—
bb	2	10	10	10	8	10	87	3
bc	—	—	—	—	—	—	1	—
EST2								
aa	1	8	1	2	2	9	72	2
ab	—	2	5	2	1	1	16	1
bb	1	—	4	6	7	—	—	—
ESTD								
bb	2	10	10	10	10	10	88	3
PEPA								
bb	2	9	10	10	10	9	81	3
bc	—	1	—	—	—	1	6	—
cc	—	—	—	—	—	—	1	—
PEPB								
ab	—	—	—	1	—	—	—	—
bb	—	7	10	5	4	—	—	—
bc	1	2	—	4	5	1	1	1
cc	1	1	—	—	1	8	82	2
cd	—	—	—	—	—	1	4	—
dd	—	—	—	—	—	—	1	—
PEPD								
aa	1	2	—	1	1	—	—	—
ab	—	3	1	2	3	—	—	—
bb	—	1	—	1	1	—	—	—
ac	—	1	—	1	—	—	—	—
bc	—	—	2	1	—	—	4	—
cc	1	—	—	—	2	9	65	2
ad	—	2	—	—	—	—	—	—
bd	—	1	4	—	1	—	—	—
cd	—	—	1	—	—	1	11	1
dd	—	—	—	—	—	—	1	—
ae	—	—	—	2	—	—	—	—
be	—	—	—	1	2	—	—	—
ce	—	—	—	1	—	—	5	—
de	—	—	2	—	—	—	—	—
ee	—	—	—	—	—	—	1	—
PEPE								
aa	2	8	9	10	8	8	80	2
ADA								
ab	—	—	1	—	—	—	1	—
bb	2	10	9	10	10	10	86	3
Lyases								
sACOH								
ab	—	2	—	1	—	—	—	—
bb	2	8	9	9	10	10	85	1
bc	—	—	—	—	—	—	3	1
mACOH								
aa	2	10	9	10	10	10	84	2

TABLE 7—(*Continued*)

Locus[b]	Site[c]							
	8 (2)	9 (10)	10 (10)	11 (10)	12 (10)	13 (10)	14 (88)	15 (3)
Isomerases								
MPI								
ab	—	—	—	—	—	—	1	—
bb	2	10	10	10	10	10	86	3
GPI								
aa	—	—	—	—	—	3	11	—
ab	1	—	—	—	—	3	32	3
bb	—	—	—	—	—	3	45	—
ac	—	—	—	1	2	—	—	—
bc	1	7	—	3	2	1	—	—
cc	—	3	10	6	6	—	—	—
PGM1								
aa	2	10	10	10	10	10	87	2
ab	—	—	—	—	—	—	1	—
PGM2								
ad	—	—	—	—	—	—	1	—
dd	2	10	10	10	10	10	87	2
PGM3								
aa	2	8	9	10	10	9	79	2
ab	—	2	1	—	—	—	5	—
bb	—	—	—	—	—	—	1	—
ac	—	—	—	—	—	1	2	—
Nonenzymic Blood Proteins								
TF								
aa	1	—	1	—	2	10	68	3
ab	—	4	1	—	4	—	3	—
bb	1	6	8	10	4	—	—	—
HB								
aa	2	10	10	10	10	10	71	3
Mitochondrial DNA								
12S mtDNA								
P	1	9	10	10	8	—	—	—
M	1	1	—	—	1	10	88	2

[a] The collecting sites associated with the northern transect are defined in table 2 and mapped in figures 3 and 4.

[b] Locus abbreviations are defined in table 5. Alleles are designated in alphabetical sequence in order of decreasing anodal migration. The 12S mtDNA data pertain to the allele-specific oligonucleotides (table 4; figs. 23 and 24); P refers to the haplotype of *C. t. punctilinealis*; M refers to the haplotype of *C. t. marmoratus.*

[c] Total lizards studied for respective sites are listed in parentheses. Where the number of observations within columns do not total the number of lizards in the sample, data were missing for one or more individuals.

TABLE 8
Frequencies of Genotypes at Collecting Sites Along the Central Transect[a]

Locus[b]	Site[c]										
	20 / 31	21 / 10	22 / 10	23 / 10	24 / 10	25 / 10	**26** / **31**	27 / 10	28 / 12	30 / 10	29 / 30
Oxidoreductases											
ADH											
aa	31	10	10	10	10	10	**31**	10	12	10	30
G3PDH											
aa	31	10	10	10	10	10	**31**	10	12	10	30
IDDH											
aa	—	—	—	—	—	—	**—**	—	—	—	4
ab	—	—	—	—	—	—	**4**	1	4	3	7
bb	31	10	10	10	10	10	**27**	9	8	7	19
LDH1											
aa	31	10	10	10	10	10	**31**	10	12	10	30
LDH2											
ab	—	—	1	—	—	—	**—**	—	—	—	—
bb	31	10	9	10	10	10	**31**	10	12	10	30
sMDH											
bb	31	9	10	10	10	10	**30**	10	12	10	30
bc	—	1	—	—	—	—	**1**	—	—	—	—
mMDH											
bb	31	10	10	10	10	10	**30**	10	12	10	30
sMDHP											
aa	—	—	—	—	—	2	**12**	1	5	6	27
ab	—	—	1	6	3	5	**13**	7	4	4	3
bb	31	9	9	4	7	3	**6**	2	2	—	—
sIDH											
ab	2	1	1	—	—	1	**1**	—	—	1	—
bb	29	9	9	10	10	9	**30**	10	12	9	30
mIDH											
aa	31	10	10	10	10	10	**31**	10	12	10	30
sSOD											
aa	31	10	10	10	10	10	**31**	10	12	10	30
mSOD											
aa	28	10	10	10	10	10	**31**	10	12	10	30
DDH											
aa	31	10	10	10	10	10	**31**	10	11	10	30
Transferases											
sAAT											
ab	2	—	—	—	—	—	**1**	—	—	—	—
bb	29	10	10	10	10	10	**30**	10	12	10	30
mAAT											
aa	1	—	—	—	—	—	**—**	—	—	—	—
ab	2	—	—	—	—	—	**—**	—	—	—	—
bb	23	10	10	8	9	10	**31**	10	12	10	30
bc	4	—	—	2	1	—	**—**	—	—	—	—
cc	1	—	—	—	—	—	**—**	—	—	—	—
AK											
aa	31	10	10	10	10	10	**31**	10	12	10	30
CK1											
bb	31	10	10	10	10	10	**31**	10	12	10	30
CK2											
aa	31	10	10	10	10	10	**31**	10	12	10	30

TABLE 8—(*Continued*)

Locus[b]	Site[c]										
	20 31	21 10	22 10	23 10	24 10	25 10	**26 31**	27 10	28 12	30 10	29 30
Hydrolases											
ALP											
aa	31	10	10	9	10	10	**31**	10	11	10	20
EST1											
ab	—	—	—	—	—	—	**—**	—	—	—	1
bb	30	9	9	10	9	10	**31**	10	12	10	27
bc	1	1	—	—	—	—	**—**	—	—	—	1
EST2											
aa	2	—	—	1	—	3	**10**	6	8	8	29
ab	3	1	3	1	3	4	**17**	3	4	2	1
bb	26	9	7	8	7	3	**4**	1	—	—	—
ESTD											
ab	1	—	—	—	—	—	**—**	—	1	—	—
bb	30	10	10	10	10	10	**31**	10	11	10	30
PEPA											
ab	—	1	—	—	—	—	**—**	—	—	—	—
bb	27	9	10	10	10	10	**24**	6	10	8	25
bc	—	—	—	—	—	—	**7**	4	—	2	4
PEPB											
bb	28	10	9	7	5	2	**6**	1	—	—	—
bc	3	—	1	3	5	6	**16**	6	2	2	2
cc	—	—	—	—	—	—	**6**	2	10	8	25
bd	—	—	—	—	—	2	**2**	—	—	—	—
cd	—	—	—	—	—	—	**1**	1	—	—	3
PEPD											
aa	17	2	4	—	—	—	**—**	—	—	—	—
ab	5	4	1	1	—	—	**4**	1	1	—	—
bb	2	—	1	2	9	1	**3**	1	—	—	—
ac	3	1	2	—	—	3	**9**	1	2	1	—
bc	2	—	1	2	—	2	**6**	1	2	2	2
cc	—	—	—	1	1	3	**6**	5	5	6	23
ad	—	—	—	2	—	—	**—**	—	—	—	—
bd	—	2	—	—	—	—	**—**	—	—	—	—
cd	—	1	—	—	—	—	**1**	—	—	—	4
ae	2	—	1	1	—	—	**—**	—	—	—	—
be	—	—	—	1	—	—	**—**	—	—	—	—
ce	—	—	—	—	—	1	**2**	1	2	1	1
PEPE											
aa	31	9	10	10	10	10	**30**	10	12	10	28
ADA											
aa	1	—	—	—	—	—	**—**	—	—	—	—
ab	2	—	—	—	—	—	**1**	—	—	—	—
bb	28	10	9	10	10	10	**30**	10	12	10	30
bc	—	—	1	—	—	—	**—**	—	—	—	—
Lyases											
sACOH											
aa	1	—	—	—	—	—	**—**	—	—	—	—
ab	5	—	—	—	—	1	**—**	—	—	—	—
bb	24	10	10	10	9	9	**31**	10	12	10	30
bc	1	—	—	—	1	—	**—**	—	—	—	—
mACOH											
aa	31	10	10	10	10	10	**31**	10	11	10	30

TABLE 8—(*Continued*)

Locus[b]	Site[c]										
	20 31	21 10	22 10	23 10	24 10	25 10	**26 31**	27 10	28 12	30 10	29 30
Isomerases											
MPI											
aa	—	—	—	—	—	—	**—**	—	—	—	1
ab	1	—	—	—	—	—	**—**	—	—	—	2
bb	30	10	10	10	10	10	**31**	10	12	10	27
GPI											
aa	—	—	—	—	—	—	**1**	1	1	2	6
ab	1	—	—	—	—	2	**9**	3	4	3	14
bb	3	1	1	4	2	1	**3**	2	3	—	10
ac	—	—	—	—	1	2	**2**	2	2	4	—
bc	14	3	6	4	6	4	**12**	1	1	1	—
cc	13	6	3	2	1	1	**4**	1	1	—	—
PGM1											
aa	31	10	10	10	10	10	**31**	10	12	10	30
PGM2											
dd	31	10	10	10	10	10	**31**	10	12	10	30
PGM3											
aa	15	6	7	9	6	8	**24**	7	11	9	27
ab	15	4	3	1	4	1	**6**	3	1	1	2
bb	—	—	—	—	—	1	**1**	—	—	—	—
bc	1	—	—	—	—	—	**—**	—	—	—	—
Nonenzymic Blood Proteins											
TF											
aa	—	—	—	—	—	1	**8**	2	5	7	30
ab	1	—	1	1	3	3	**13**	7	4	3	—
bb	29	10	9	9	7	6	**9**	1	3	—	—
HB											
aa	31	10	10	10	10	10	**30**	10	12	10	30
Mitochondrial DNA											
12S mtDNA											
P	28	10	10	6	6	4	**10**	2	1	—	—
M	—	—	—	4	4	6	**21**	8	11	10	30

[a] The collecting sites and central transect are defined in table 2 and mapped in figures 3 and 5. Site 26 is the 50:50 point of gene exchange (midpoint).

[b] Locus abbreviations are defined in table 5. Alleles for protein loci are designated in alphabetical sequence in order of decreasing anodal migration. The 12S mtDNA data pertain to the allele-specific oligonucleotides (table 4; figs. 23 and 24); P refers to the haplotype of *C. t. punctilinealis*; M refers to the haplotype of *C. t. marmoratus*.

[c] Total lizards studied for respective sites are listed in parentheses. Where the number of observations within columns do not total the number of lizards in the sample, data were missing for one or more individuals.

TABLE 9
Frequencies of Genotypes at Collecting Sites Associated with the Central Transect[a]

Locus[b]	16 (10)	17 (1)	18 (10)	19 (10)	31 (10)
Oxidoreductases					
ADH					
aa	10	1	10	10	10
G3PDH					
aa	10	1	10	10	10
IDDH					
aa	1	—	1	—	—
ab	4	—	1	—	5
bb	5	1	8	10	5
LDH1					
aa	10	1	10	10	10
LDH2					
bb	10	1	10	10	10
sMDH					
bb	10	1	10	10	10
mMDH					
bb	10	1	10	10	10
sMDHP					
aa	10	1	3	2	8
ab	—	—	3	6	2
bb	—	—	4	2	—
sIDH					
ab	1	—	—	1	—
bb	9	1	10	9	10
mIDH					
aa	10	1	10	10	10
sSOD					
aa	3	1	10	10	10
mSOD					
aa	10	1	10	10	10
DDH					
aa	10	1	10	10	10
Transferases					
sAAT					
bb	10	1	10	10	10
mAAT					
bb	10	1	10	9	10
bc	—	—	—	1	—
AK					
aa	10	1	10	10	10
CK1					
bb	10	1	10	9	10
CK2					
aa	10	1	10	10	10
Hydrolases					
ALP					
aa	10	1	6	10	10
EST1					
bb	10	1	9	10	10

TABLE 9—(*Continued*)

Locus[b]	16 (10)	17 (1)	18 (10)	19 (10)	31 (10)
EST2					
aa	10	1	8	2	8
ab	—	—	1	6	2
bb	—	—	1	2	—
ESTD					
ab	1	—	1	—	—
bb	9	1	9	10	10
PEPA					
ab	1	—	—	—	—
bb	9	1	9	10	8
bc	—	—	1	—	2
PEPB					
bb	—	—	1	2	—
bc	—	—	2	6	1
cc	8	1	7	1	8
bd	—	—	—	1	1
cd	2	—	—	—	—
PEPD					
aa	—	—	—	1	—
ab	—	—	—	2	—
bb	—	—	—	1	—
bc	1	—	1	4	1
cc	8	1	—	—	7
ad	—	—	1	—	—
bd	—	—	1	—	—
cd	—	—	2	—	—
dd	—	—	1	—	—
ae	—	—	3	—	1
ce	1	—	1	2	1
PEPE					
aa	9	1	10	10	10
ADA					
ab	2	—	—	1	—
bb	8	1	10	9	10
Lyases					
sACOH					
bb	10	1	8	10	9
bc	—	—	—	—	1
mACOH					
aa	10	1	4	10	10
Isomerases					
MPI					
bb	10	1	10	10	10
GPI					
aa	1	—	3	—	4
ab	8	—	3	1	3
bb	1	—	2	—	2
ac	—	1	2	2	1
bc	—	—	—	3	—
cc	—	—	—	4	—

TABLE 9—(*Continued*)

Locus[b]	Site[c]				
	16 (10)	17 (1)	18 (10)	19 (10)	31 (10)
PGM1					
aa	9	1	10	10	10
PGM2					
dd	10	1	10	10	10
Pgm3					
aa	7	1	7	10	10
ab	3	—	3	—	—
Nonenzymic Blood Proteins					
TF					
aa	9	1	6	3	9
ab	1	—	4	4	1
bb	—	—	—	3	—
HB					
aa	10	1	10	10	10
Mitochondrial DNA					
12S mtDNA					
P	—	—	—	1	—
M	10	1	10	8	10

[a] The collecting sites associated with the central transect are defined in table 2 and mapped in figures 3 and 5.

[b] Locus abbreviations are defined in table 5. Alleles of protein loci are designated in alphabetical sequence in order of decreasing anodal migration. The 12S mtDNA data pertain to the allele-specific oligonucleotides (table 4; figs. 23 and 24); P refers to the haplotype of *C. t. punctilinealis*; M refers to the haplotype of *C. t. marmoratus*.

[c] Total lizards studied for respective sites are listed in parentheses. Where the number of observations within columns do not total to the number of lizards in the sample, data were missing for one or more individuals.

sects, and table 12 gives such data for samples of *C. t. punctilinealis* and *C. t. marmoratus* from sites distant from the contact region. For organizational purposes, we discuss the 36 loci in groups based on levels of genetic diversity.

MONOMORPHIC LOCI (BUT WITH SOME UNCOMMON ALLELES): Eleven loci were literally invariant (ADH, LDH1, sSOD, mSOD, DDH, AK, CK2, ALP, PEPE, mACOH, and HB), and twelve exhibited variants of very low frequency only (G3PDH, LDH2, sMDH, mMDH, mIDH, sAAT, CK1, ESTD, ADA, MPI, PGM1, and PGM2; table 22; see "Uncommon Alleles" below). Alleles at three of these loci, ADH, ESTD, and PGM2, were unique to specimens of *C. t. septentrionalis*

or *C. t. aethiops* (table 31 and fig. 51; see "Comparisons With Additional Subspecies of *C. tigris*" below).

POLYMORPHIC LOCI: Thirteen loci showed relatively high frequencies of multiple alleles. Seven of these loci were highly polymorphic, including alleles that distinguished the two subspecies in the contact region (IDDH, sMDHP, EST2, PEPB, PEPD, GPI, and TF). Four of these—EST2 (fig. 25), sMDHP and PEPB (fig. 28), and TF (fig. 27)—we define as diagnostic loci, as the interbreeding subspecies were fixed or nearly fixed for alternative alleles at these four loci (tables 13–15). Alleles diagnostic of each subspecies clearly delineated clines of genetic variation across the three transects. Polymorphism was relatively low at most sites for the six remaining loci (sIDH, mAAT, EST1, PEPA, sACOH, and PGM3 [fig. 26]).

Although allele frequencies at IDDH, PEPD, and GPI differed in samples of the pure subspecies (table 14), we do not include them as diagnostic loci. The b-allele of IDDH (fig. 27) occurred with high frequency in both subspecies; however, the a-allele was unique to *marmoratus*, although its maximum frequency at any site was 0.65. PEPD and GPI (fig. 29) also had alleles that were present in both subspecies, but they had certain alleles with clearly the highest frequencies in one or the other subspecies: the a- and b-alleles of PEPD had their highest frequencies in *punctilinealis*, and the c-allele of PEPD was most frequent in *marmoratus*. The c-allele of GPI had a high frequency in *punctilinealis*, but the a- and b-alleles of GPI were highest in *marmoratus*. Appendix 3 lists genotypes at the 13 polymorphic loci and the haplotypes of 12S ribosomal mtDNA for each of the 614 individuals from the 50 collecting sites in or near the contact region.

FEATURES OF THE THREE HYBRID ZONES

Samples forming a transect were collected across each of three hybrid zones (northern, central, and southern; figs. 4 and 5). Considering the topography and ecology involved (fig. 3), each of these probably originated as separate and independent hybrid zones, although today the central and southern hybrid zones are probably blending in the vicinity

TABLE 10
Frequencies of Genotypes at Collecting Sites Along the Southern Transect[a]

Locus[b]	Site[c]								
	36 (10)	37 (10)	38 (10)	39 (10	40 (10)	(**42**) (**10**)	44 (10)	46 (10)	48 (10)
				Oxidoreductases					
ADH									
aa	10	10	10	10	10	**10**	10	10	10
G3PDH									
aa	10	10	10	9	10	**10**	10	10	10
ab	—	—	—	1	—	**—**	—	—	—
IDDH									
aa	—	—	—	—	—	**—**	—	—	1
ab	—	—	—	—	1	**2**	3	2	6
bb	10	10	10	10	9	**8**	7	8	3
LDH1									
aa	10	10	10	10	10	**10**	10	10	10
LDH2									
bb	10	10	10	10	10	**10**	10	10	10
sMDH									
bb	10	10	10	10	10	**10**	10	10	10
mMDH									
ab	—	—	—	—	—	**—**	—	—	1
bb	10	10	10	10	10	**10**	10	10	9
sMDHP									
aa	—	—	—	—	—	**1**	5	9	10
ab	1	1	—	1	5	**7**	2	1	—
bb	9	9	10	9	5	**2**	3	—	—
sIDH									
ab	1	1	—	1	1	**—**	1	—	—
bb	9	9	10	9	9	**10**	9	10	10
mIDH									
aa	10	10	10	10	10	**10**	10	10	9
ab	—	—	—	—	—	**—**	—	—	1
sSOD									
aa	10	10	10	10	10	**10**	10	10	10
mSOD									
aa	10	10	10	10	10	**10**	10	10	10
DDH									
aa	10	10	10	10	10	**10**	10	10	10
				Transferases					
sAAT									
bb	10	10	10	10	10	**10**	10	10	10
mAAT									
ab	—	—	1	—	—	**—**	—	—	—
bb	6	8	8	7	8	**8**	10	10	10
bc	3	2	1	3	2	**2**	—	—	—
cc	1	—	—	—	—	**—**	—	—	—
AK									
aa	10	10	10	10	10	**10**	10	10	10
CK1									
bb	10	10	10	10	10	**10**	10	10	10
CK2									
aa	10	10	10	10	10	**10**	10	10	10

TABLE 10—(*Continued*)

Locus[b]	Site[c]								
	36 (10)	37 (10)	38 (10)	39 (10	40 (10)	**(42)** **(10)**	44 (10)	46 (10)	48 (10)
	Hydrolases								
ALP									
aa	9	10	10	10	10	**10**	10	10	10
EST1									
ab	—	—	2	1	—	**—**	—	—	—
bb	6	10	8	9	8	**10**	8	10	10
bc	4	—	—	—	—	**—**	2	—	—
EST2									
aa	—	—	1	1	1	**8**	8	8	6
ab	3	1	3	3	3	**2**	2	2	4
bb	7	9	6	6	6	**—**	—	—	—
ESTD									
bb	10	10	10	10	10	**10**	10	10	10
PEPA									
aa	—	—	1	—	—	**—**	—	—	—
ab	—	—	—	—	—	**1**	—	—	—
bb	10	10	9	10	9	**8**	10	7	9
bc	—	—	—	—	1	**—**	—	3	1
cc	—	—	—	—	—	**1**	—	—	—
PEPB									
bb	10	10	10	10	8	**6**	2	—	—
bc	—	—	—	—	2	**4**	5	2	1
cc	—	—	—	—	—	**—**	3	7	7
cd	—	—	—	—	—	**—**	—	1	1
dd	—	—	—	—	—	**—**	—	—	1
PEPD									
aa	7	3	2	1	1	**3**	—	—	—
ab	2	6	2	—	1	**2**	1	—	1
bb	—	—	1	—	1	**1**	1	—	—
ac	—	1	2	5	3	**2**	4	1	1
bc	1	—	—	2	2	**—**	1	—	—
cc	—	—	—	1	—	**1**	3	7	8
ad	—	—	2	—	2	**—**	—	—	—
bd	—	—	—	1	—	**—**	—	—	—
ae	—	—	1	—	—	**—**	—	—	—
be	—	—	—	—	—	**—**	—	1	—
ce	—	—	—	—	—	**1**	—	—	—
ee	—	—	—	—	—	**—**	—	1	—
PEPE									
aa	10	7	10	10	10	**10**	10	10	10
ADA									
bb	10	10	10	10	10	**10**	10	10	10
	Lyases								
sACOH									
ab	3	1	2	—	1	**1**	—	—	—
bb	7	8	8	10	9	**9**	10	10	10
bc	—	1	—	—	—	**—**	—	—	—
mACOH									
aa	10	10	10	10	10	**10**	10	10	10

TABLE 10—(*Continued*)

	Site[c]								
Locus[b]	36 (10)	37 (10)	38 (10)	39 (10	40 (10)	(**42**) (**10**)	44 (10)	46 (10)	48 (10)
Isomerases									
MPI									
bb	10	10	10	10	10	**10**	10	10	10
GPI									
aa	—	—	—	—	—	**—**	1	1	2
ab	—	—	—	—	—	**1**	3	3	7
bb	—	—	1	1	1	**3**	—	3	1
ac	—	—	—	—	1	**2**	1	1	—
bc	4	1	1	5	5	**1**	2	2	—
cc	6	9	8	4	3	**3**	3	—	—
PGM1									
aa	10	10	10	10	10	**10**	10	10	10
PGM2									
dd	10	10	10	10	10	**10**	10	10	10
PGM3									
aa	6	6	7	6	7	**9**	6	10	10
ab	4	4	3	4	3	**—**	3	—	—
bb	—	—	—	—	—	**1**	1	—	—
Nonenzymic Blood Proteins									
TF									
aa	—	—	—	—	—	**2**	2	7	10
ab	—	—	—	—	2	**5**	7	3	—
bb	10	10	10	10	8	**3**	1	—	—
HB									
aa	10	10	10	10	10	**10**	10	10	10
Mitochondrial DNA									
12S mtDNA									
P	10	10	10	10	8	**2**	1	—	1
M	—	—	—	—	2	**8**	9	10	9

[a] The collecting sites and southern transect are defined in table 2 and mapped in figures 3 and 5. Site 42 is the 50:50 point of gene exchange (midpoint).

[b] Locus abbreviations are defined in table 5. Alleles for protein loci are designated in alphabetical sequence in order of decreasing anodal migration. The 12S mtDNA data pertain to the allele-specific oligonucleotides (table 4; figs. 23 and 24); P refers to the haplotype of *C. t. punctilinealis*; M refers to the haplotype of *C. t. marmoratus*.

[c] Total lizards for respective sites are listed in parentheses. Where the number of observations within columns do not total the number of lizards in the sample, data were missing for one or more individuals.

TABLE 11
Frequencies of Genotypes at Collecting Sites Associated with the Southern Transect[a]

Locus[b]	Site[c]									
	32 10	33 10	34 10	35 10	41 10	43 10	45 10	47 10	50 2	51 5
					Oxidoreductases					
ADH										
aa	10	10	10	10	10	10	10	10	2	5
G3PDH										
aa	10	10	10	10	10	10	10	10	2	5
IDDH										
aa	1	—	—	—	—	—	—	2	—	—
ab	2	3	2	—	1	—	4	4	—	—
bb	7	7	8	10	9	10	6	4	2	5
LDH1										
aa	10	10	10	10	10	10	10	10	2	5
LDH2										
bb	10	10	10	10	10	10	10	10	2	5
sMDH										
bb	10	10	10	10	10	10	10	10	2	5
mMDH										
bb	10	10	10	10	10	10	10	10	2	5
sMDHP										
aa	7	5	—	—	2	—	6	10	—	—
ab	2	3	3	3	3	7	4	—	—	3
bb	1	2	7	7	5	3	—	—	2	2
sIDH										
ab	—	—	—	—	1	—	—	—	—	—
bb	10	10	10	10	9	10	10	10	2	5
mIDH										
aa	10	10	10	10	10	10	10	10	2	5
sSOD										
aa	10	10	10	10	10	10	10	10	2	5
mSOD										
aa	10	10	10	10	10	10	10	10	2	5
DDH										
aa	10	10	10	10	10	10	10	10	2	5
					Transferases					
sAAT										
ab	—	—	—	—	—	2	—	—	—	—
bb	10	10	10	10	10	8	10	10	2	5
mAAT										
bb	10	10	7	9	10	10	10	10	2	3
bc	—	—	3	1	—	—	—	—	—	2
AK										
aa	10	10	10	10	10	10	10	10	1	5
CK1										
bb	10	10	10	10	10	10	10	10	1	5
CK2										
aa	10	10	10	10	10	10	10	10	2	5
					Hydrolases					
ALP										
aa	9	10	10	10	10	10	10	10	2	5
EST1										
bb	10	10	8	10	10	10	8	10	2	5
bc	—	—	2	—	—	—	2	—	—	—

TABLE 11—(*Continued*)

Locus[b]	Site[c]									
	32 10	33 10	34 10	35 10	41 10	43 10	45 10	47 10	50 2	51 5
EST2										
aa	10	6	1	3	4	—	7	10	—	—
ab	—	2	2	5	3	3	3	—	—	2
bb	—	1	7	2	3	7	—	—	2	3
bc	—	1	—	—	—	—	—	—	—	—
ESTD										
bb	10	10	10	10	10	10	10	10	2	5
PEPA										
bb	5	4	9	10	10	8	6	10	2	5
bc	4	4	1	—	—	2	4	—	—	—
cc	1	2	—	—	—	—	—	—	—	—
PEPB										
bb	—	—	6	7	6	3	1	—	2	4
bc	—	4	3	1	2	6	3	2	—	1
cc	9	6	1	2	2	1	6	4	—	—
cd	1	—	—	—	—	—	—	4	—	—
PEPD										
aa	—	1	2	2	1	4	1		1	1
ab	—	—	1	3	—	2	—	—	1	—
bb	—	—	1	1	—	—	—	—	—	—
ac	—	1	2	2	4	2	—	1	—	1
bc	1	1	2	1	1	—	—	—	—	2
cc	6	6	1	—	4	1	—	9	—	1
ad	—	1	—	—	—	—	2	—	—	—
dd	—	—	—	—	—	—	6	—	—	—
ae	—	—	1	—	—	—	—	—	—	—
be	—	—	—	1	—	1	—	—	—	—
ce	3	—	—	—	—	—	1	—	—	—
PEPE										
aa	10	10	10	10	10	10	10	10	2	5
ADA										
bb	10	10	10	10	10	10	10	10	2	5
Lyases										
sACOH										
ab	—	—	2	—	—	—	—	—	—	—
bb	10	10	8	10	10	10	10	10	2	5
mACOH										
aa	10	10	10	10	10	10	10	10	2	4
Isomerases										
MPI										
ab	—	—	—	—	—	—	—	—	1	—
bb	10	10	10	10	10	10	10	10	1	5
GPI										
aa	6	2	—	—	1	1	2	—	—	—
ab	1	2	—	—	—	2	4	8	—	—
bb	2	3	2	—	1	—	1	2	—	—
ac	1	1	2	1	1	1	2	—	—	—
bc	—	2	2	5	4	5	1	—	—	1
cc	—	—	4	4	3	1	—	—	2	4

TABLE 11—(*Continued*)

Locus[b]	Site[c]									
	32 10	33 10	34 10	35 10	41 10	43 10	45 10	47 10	50 2	51 5
PGM1										
aa	10	10	10	10	10	10	9	10	2	5
PGM2										
dd	10	10	10	10	10	10	10	10	2	5
PGM3										
aa	10	10	8	9	9	8	10	10	2	4
ab	—	—	2	—	1	1	—	—	—	1
bb	—	—	—	1	—	1	—	—	—	—
Nonenzymic Blood Proteins										
TF										
aa	8	8	—	—	1	—	8	10	—	—
ab	2	2	3	2	5	4	2	—	—	—
bb	—	—	7	8	4	6	—	—	2	5
HB										
aa	10	10	10	10	10	10	10	10	2	5
Mitochondrial DNA										
12S mtDNA										
P	—	—	8	7	7	6	—	—	2	5
M	10	10	2	3	3	4	10	10	—	—

[a] The collecting sites associated with the southern transect are defined in table 2 and mapped in figures 3 and 5. See appendix 2 for location of sites 50 and 51.

[b] Locus abbreviations are defined in table 5. Alleles for protein loci are designated in alphabetical sequence in order of decreasing anodal migration. The 12S mtDNA data pertain to the allele-specific oligonucleotides (table 4; figs. 23 and 24); P refers to the haplotype of *C. t. punctilinealis*; M refers to the haplotype of *C. t. marmoratus*.

[c] Total lizards studied for respective sites are listed in parentheses. Where the number of observations within columns do not total the number of lizards in the sample, data were missing for one or more individuals.

TABLE 12

Frequencies of Genotypes at Collecting Sites Distant from the Contact Region[a]

	Site[c]				
	punctilinealis		*marmoratus*		
Locus[b]	49 (11)	56 (1)	52 (10)	53 (8)	54 (10)
Oxidoreductases					
ADH					
aa	6	—	10	8	10
ab	5	1	—	—	—
G3PDH					
aa	11	1	8	8	10
ab	—	—	2	—	—
IDDH					
aa	—	—	1	3	3
ab	—	—	8	1	5
bb	11	1	1	4	2
LDH1					
aa	11	1	10	8	10
LDH2					
bb	11	1	10	8	10
sMDH					
bb	11	1	10	8	10
mMDH					
bb	11	1	10	7	10
bc	—	—	—	1	—
sMDHP					
aa	9	1	10	8	10
ab	2	—	—	—	—
sIDH					
ab	—	—	1	—	—
bb	11	1	9	8	10
mIDH					
aa	11	—	10	8	10
sSOD					
aa	11	1	10	8	10
mSOD					
aa	11	1	10	8	10
DDH					
aa	11	1	10	8	10
Transferases					
sAAT					
a+b[d]	1	—	—	—	—
bb	8	1	10	8	10
bc	2	—	—	—	—
mAAT					
bb	11	1	10	8	10
AK					
aa	11	1	10	8	10
CK1					
bb	10	1	10	8	10
CK2					
aa	11	1	10	8	10

TABLE 12—(*Continued*)

	Site[c]				
	punctilinealis		*marmoratus*		
Locus[b]	49 (11)	56 (1)	52 (10)	53 (8)	54 (10)
Hydrolases					
ALP					
aa	8	1	10	8	7
EST1					
bb	11	1	10	8	9
bc	—	—	—	—	1
EST2					
aa	4	1	9	8	10
ab	2	—	—	—	—
bb	2	—	—	—	—
ac	1	—	—	—	—
bc	2	—	—	—	—
ESTD					
bb	11	1	10	8	10
PEPA					
bb	11	—	6	2	5
bc	—	1	4	5	5
cc	—	—	—	1	—
PEPB					
ab	4	—	—	—	—
ac	1	—	—	—	—
bb	4	1	—	—	—
bc	1	—	1	—	—
cc	1	—	7	8	10
cd	—	—	2	—	—
PEPD					
bb	11	1	—	—	—
bc	—	—	—	1	2
cc	—	—	3	—	5
cd	—	—	1	5	1
ce	—	—	5	2	2
ee	—	—	1	—	—
PEPE					
aa	11	—	10	8	8
ADA					
bb	11	1	10	8	10
Lyases					
sACOH					
bb	10	1	10	8	10
mACOH					
aa	10	—	10	8	10
Isomerases					
MPI					
bb	11	1	10	8	10
GPI					
aa	—	—	4	6	7
ab	3	—	5	2	1
bb	8	1	1	—	2

TABLE 12—(*Continued*)

Locus[b]	Site[c]				
	punctilinealis		*marmoratus*		
	49 (11)	56 (1)	52 (10)	53 (8)	54 (10)
PGM1					
aa	11	1	10	8	10
PGM2					
ad	2	—	—	—	—
dd	9	1	10	8	10
PGM3					
aa	5	1	10	8	6
ab	2	—	—	—	2
bb	—	—	—	—	1
ac	2	—	—	—	—
Nonenzymic Blood Proteins					
TF					
aa	—	—	10	—	10
ab	1	—	—	—	—
bb	10	1	—	—	—
HB					
aa	11	1	10	—	10
Mitochondrial DNA					
12S mtDNA					
P_t	11	1	—	—	—
M	—	—	10	8	10

[a] The location of the collecting sites for these *punctilinealis* and *marmoratus* are described in appendix 2 and mapped in figure 1.

[b] Locus abbreviations are defined in table 5. Alleles of protein loci are designated in alphabetical sequence in order of decreasing anodal migration. The 12S mtDNA data pertain to the allele-specific oligonucleotides (table 4; figs. 23 and 24); P_t designates the haplotype found in *C. t. punctilinealis* from localities west of the contact region (Dessauer et al., 1996b); M designates the haplotype of *C. t. marmoratus.*

[c] Total lizards studied for respective sites are listed in parentheses. Where the number of observations within columns do not total the number of lizards in the sample, data were missing for one or more individuals.

[d] The product of the a[+]-allele at sAAT migrated faster than that of the a-allele found in a few other specimens of *C. tigris* (e.g., at site 20).

of sites 31 and 32 (fig. 49). Plots of gene frequencies against distance along each transect followed almost identical patterns of change (figs. 30–32). Each plot revealed steep step-clines in frequencies of *marmoratus* mtDNA haplotypes and *marmoratus* nuclear alleles. Clines for mtDNA and each protein locus were congruent and coincident,
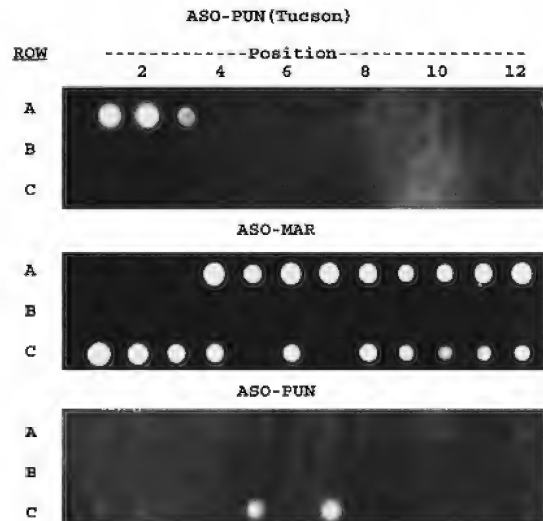

Fig. 23. Dot-blot illustrating specificity of the allele-specific oligonucleotide probes (ASOs). DNA samples from 24 lizards from 12 different sites were applied in rows A and C of a strip of nitrocellulose paper. After heat denaturation, the blot was hybridized successively with ASO-PUN (Tucson) (**Top**), ASO-Mar (**Middle**), and ASO-PUN (**Bottom**). Note that *C. t. punctilinealis* samples from sites 49 and 56, west of the contact region, hybridized only to ASO-PUN (Tucson) (positions 1–3 of row A), in contrast to samples of *punctilinealis* from the contact region, which hybridized only with ASO-PUN (positions 5 and 7 of row C). DNA of *C. t. marmoratus* from all sites paired with ASO-MAR (positions 4–12 of row A, and positions 1–4, 6, and 8–12 of row C).

with steep steps in allele frequencies centered close to the same position along the transect. Cline widths, defined as the distance between sites with allele frequencies between 0.2 and 0.8 (May et al., 1975), were between three and 12 km for each of the four diagnostic loci and the mtDNA haplotypes in each transect. However, diagnostic alleles for each subspecies penetrated the geographic range of the opposite taxon over a considerably broader area, as discussed below. The center (midpoint) of each cline was the site where diagnostic allele frequencies approximated 0.5 and the rate of change of allele frequencies with distance (slope) was greatest (figs. 30–32).

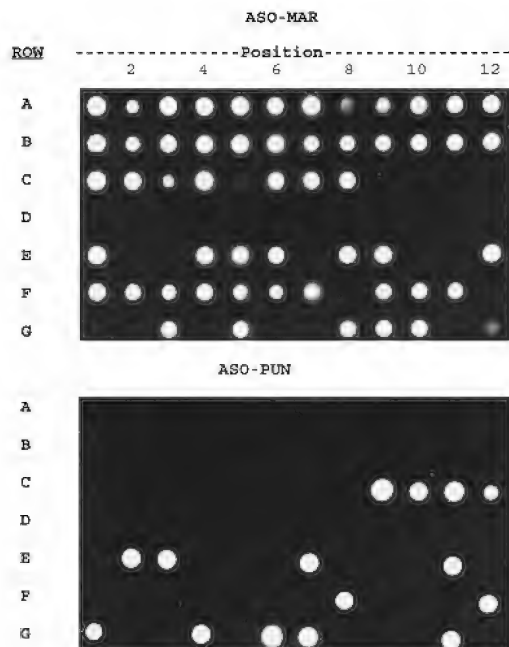NORTHERN TRANSECT (fig. 30): The northern transect included seven sites (1–7) along

Fig. 24. Dot-blot illustrating determination of the 12S ribosomal mtDNA haplotypes of individual lizards from 20 collecting sites across the contact region. The blot was first hybridized with the ASO-MAR probe specific for the *marmoratus* haplotype (**Top**). After stripping, the blot was hybridized with the ASO-PUN probe specific for the *punctilinealis* haplotype (**Bottom**). Spillage caused the absence of reactions at positions E10 and G2. DNA of *C. inornatus* gave the weak positive reaction at position G12 (**Top**), as discussed by Dessauer et al. (1996b).

Highway 464 between Redrock, New Mexico, and Lordsburg (table 6; fig. 4). Eight associated sites off the transect contributed to assessments of diversity for the northern region (table 7). With the exception of some irregularity in the distribution of the a-allele of sMDHP, step-clines of the mtDNA and allozyme loci were symmetrical. The hybrid zone was about eight km wide and was centered close to site 3 (32°36'10.0″N, 108°44'17.8″W).

CENTRAL TRANSECT (fig. 31): The central transect funneled from west to east through Steins Pass in the Peloncillo Mountains. The 11 sites (20–30 and 29) paralleled a pipeline road immediately south of Interstate Highway 10 (table 8, fig. 5). Five associated sites

off the transect contributed to assessments of diversity for the central region (table 9). Distributions of the mtDNA haplotypes and nuclear alleles were the most symmetrical of the three transects. The width of the hybrid zone was about three km (the narrowest of the three transects) and the center was close to site 26 (32°13'24.6″N, 108°57'51.8″W). We have presented a preliminary report on this transect (Dessauer and Cole, 1991).

SOUTHERN TRANSECT (fig. 32): The southern transect included nine collecting sites (36–40 plus 42, 44, 46, and 48) extending eastward from the center of the San Simon Valley, Arizona, across the Arizona–New Mexico boundary, through the Peloncillo Mountains north of Granite Gap, and eastward toward Cotton City, New Mexico (table 10, fig. 5). Eight associated sites off the transect contributed to assessments of diversity for the southern region (table 11, which also includes sites 50 and 51). Step-clines of allozyme frequencies, especially that of EST2, and mtDNA haplotypes were somewhat less concordant relative to the other transects. The hybrid zone was about 5.5 km wide and its center was close to site 42, approximately 1 km east of Granite Gap (32°5'35.4″N, 108°56'50.8″W).

INDICES OF DIVERSITY WITHIN THE CONTACT REGION

Lizards within the contact region exhibit a great variety of genotypes (table 13). Considering the four diagnostic loci alone, genotypes of individual lizards were observed with 91 of the 162 possible combinations of alleles (product of number of possible genotypes at each locus: 3 for sMDHP $\times$ 3 for EST2 $\times$ 6 for PEPB $\times$ 3 for TF). The most common combinations of alleles were those characteristic of pure *punctilinealis* and pure *marmoratus* from outside the hybrid zones (table 14). No lizard with combinations of genes typical of either pure subspecies crossed the center of any of the step-clines (table 13). Possible F_1 hybrids (heterozygous at each diagnostic locus) were rare at all sites; only five were found among lizards from the transect sites (table 13) and only
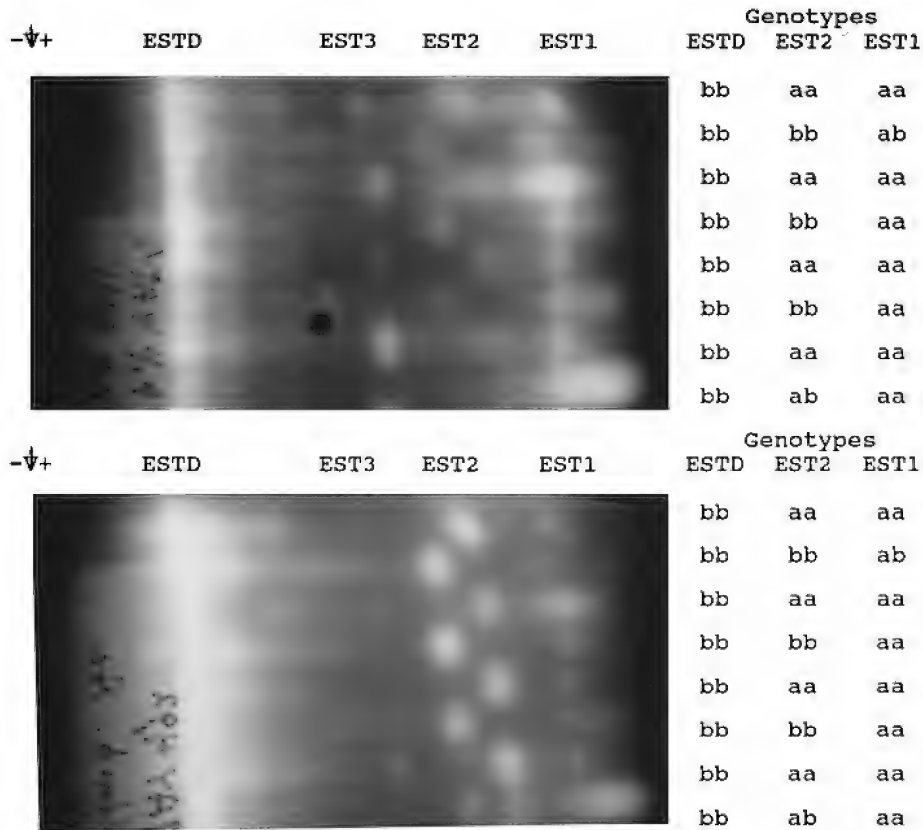
Fig. 25.   Identification of esterase loci illustrating the diversity of diagnostic locus EST2 in muscle homogenates. **Top**. Gel stained with 4-methyl-umbelliferyl butyrate, a substrate that enhances the activity of EST1 and minimizes that of EST2. **Bottom**. Opposite half of the same gel stained with 4-methyl-umbelliferyl acetate, a substrate that enhances the activity of EST2. The a-allele of EST2 is diagnostic for *marmoratus*; the b-allele is diagnostic for *punctilinealis*. Note that alleles for EST1 are mislabeled, owing to discovery of the rare a-allele (table 22) after labeling of this figure. Therefore, in this figure read the a-allele of EST1 as b, and read the b-allele as c, to be consistent with table 6. These fluorescent banding patterns were photographed in ultraviolet light; arrows indicate sites of sample application; anode is to the right.

three among specimens from associated sites. The hybrids clearly were fertile, as the great majority of specimens from intermediate sites along the transects had genotypes characteristic of backcrosses and hybrids of generations beyond the F_1. Diversity of hybrid genomes reached a maximum at sites near the center of each transect (table 13).

Indices of diversity varied clinally across each transect (table 15). The percentage of *marmoratus* alleles at the four diagnostic loci increased from about 5% at pure *punctilinealis* sites (sites 1, 20, and 36) at one end of each transect to about 95% at pure *marmoratus* sites at the other end of each transect

(sites 7, 29, and 48; in this paper we use the word "pure" in a relative rather than an absolute sense). Mean heterozygosities for the four diagnostic loci ranged from 0.2 or less for pure *punctilinealis* or pure *marmoratus* to maxima of from 0.285 to 0.323 for sites at or near the center of each transect (the highest H was at sites 4, 27, 26, 25, and 44). Nei and Hillis genetic distances from pure *marmoratus* dropped progressively across the transects from approximately 0.5 for samples from pure *punctilinealis* sites to 0 for samples from pure *marmoratus* sites, based on the 13 polymorphic loci (table 15, footnote b).
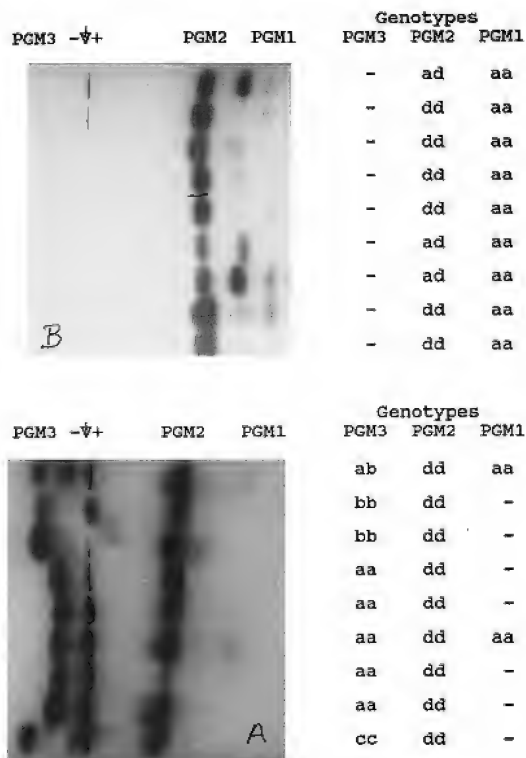
				Genotypes		
PGM3	−▼+	PGM2	PGM1	PGM3	PGM2	PGM1
				−	ad	aa
				−	dd	aa
				−	dd	aa
				−	dd	aa
				−	dd	aa
				−	ad	aa
				−	ad	aa
				−	dd	aa
B				−	dd	aa

				Genotypes		
PGM3	−▼+	PGM2	PGM1	PGM3	PGM2	PGM1
				ab	dd	aa
				bb	dd	−
				bb	dd	−
				aa	dd	−
				aa	dd	−
				aa	dd	aa
				aa	dd	−
				aa	dd	−
A				cc	dd	−

Fig. 26. Differences in the tissue distribution of phosphoglucomutases. **Top**. Patterns obtained with homogenates of skeletal muscle in which PGM1 and PGM2 are active but PGM3 is not. **Bottom**. Patterns obtained with homogenates of liver in which PGM2 and PGM3 are active but PGM1 is inactive or of very low activity. Arrows indicate sites of sample application; anode is to the right.

HARDY-WEINBERG DISEQUILIBRIUM TESTS

Do genotype frequencies at sites across the contact region reflect simply the product of allele frequencies for variable loci in *C. tigris*, or are factors such as selection at play? To seek answers to this question, Chi-square tests for Hardy-Weinberg disequilibrium were performed for each of the seven highly polymorphic loci, using specimens from sites for which the sample size was 10 or more (table 16). Two hundred ninety-three of the 315 tests were possible; the 22 impossible tests were for sites of nonhybrids having loci with no alternative alleles.

All seven loci were in Hardy-Weinberg equilibrium at 36 of the 45 sites tested; only one of the seven loci was indicated as devi-

ating significantly from equilibrium ($P < 0.05$) at each of the other nine sites. Each of the nine instances apparently not in Hardy-Weinberg equilibrium (table 16) showed a deficiency of heterozygotes, with no homozygote in particular excess over the other. However, four of these examples involved PEPD and GPI, both of which are most polymorphic and consequently most prone to error owing to small sample sizes. Of the remaining five examples (2% of the 293 tests), three of the P values rounded to 0.05 (table 16). No locus showed consistent deviations at various sites across the contact region. Additionally, all global tests indicated Hardy-Weinberg equilibrium at each locus for pooled data for each transect and its associated sites.

It is within the limits of probable experimental error to conclude that each locus at each of the 45 sites tested is in Hardy-Weinberg equilibrium. The overall data suggest that mating throughout the contact region occurs at random and that genotype frequencies are largely undisturbed by selection, drift, mutation, or migration.

LINKAGE DISEQUILIBRIUM TESTS

Are there pairs of loci at sites close to the centers of the *Cnemidophorus tigris* hybrid zones that are in linkage (genotypic) disequilibrium? Locus pairs in disequilibrium, considered as evidence for selection against hybrids or assortative mating, if not owing to tight linkage or dispersal, has been observed in many other studies of hybrid zones (Barton and Hewitt, 1985, 1989; Szymura and Barton, 1991). To examine this possibility, we carried out linkage disequilibrium analyses on genotypes of all combinations of pairs of the seven highly polymorphic loci (table 17) for all sites with 10 or more specimens. In addition, tests were carried out with pooled data for each transect and its associated sites (table 17).

Fisher tests for each site and for combinations of sites were performed without shuffling genotypes (table 17). Seventy-four percent of the tests on individual sites were possible (699 of 945 total). Most locus pairs that were not possible to test were from sites with low heterozygosities. Only 13 locus pairs (13
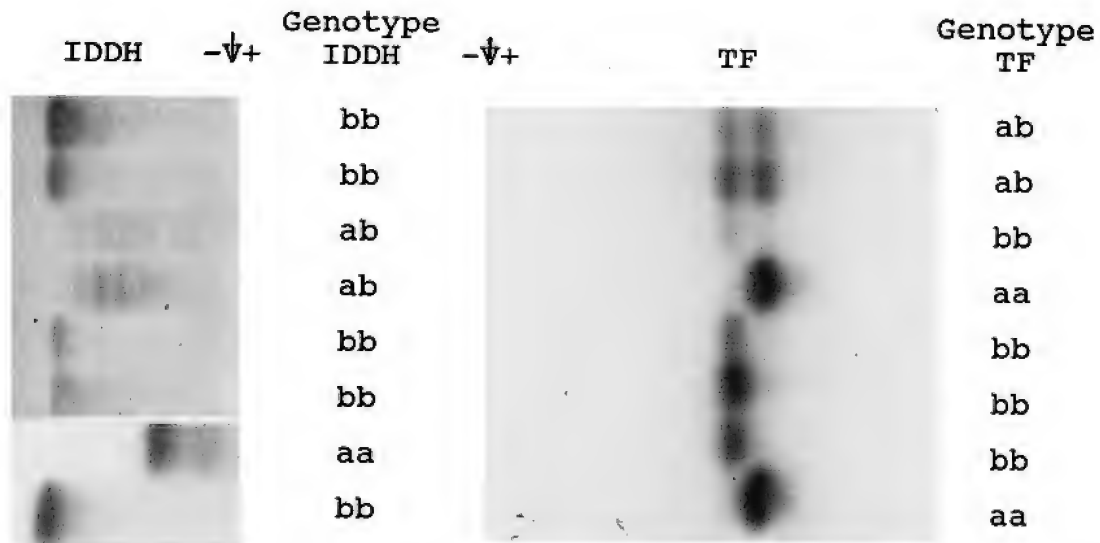
Fig. 27. Banding patterns of iditol dehydrogenase (IDDH) and diagnostic locus transferrin (TF). **Left**. Gel stained for IDDH. The b-allele occurs in both subspecies; the IDDH a-allele is diagnostic for *marmoratus*, in which it occurs exclusively (excepting hybrids), but at a maximum frequency of about 0.65. **Right**. Autoradiograph illustrating the localization of TF by binding of radioactive ^{59}Fe. The TF a-allele is diagnostic for *marmoratus*, the b-allele for *punctilinealis*. Arrows indicate sites of sample application; anode is to the right.

of 699, or 2%) distributed at nine individual sites were apparently in linkage disequilibrium; only the combinations PEPD-GPI, sMDHP-GPI, and sMDHP-TF occurred twice (table 17). Note also that for eight of these 13 pairs, PEPD or GPI or both were involved. As discussed above, these loci show the highest polymorphism and consequently the greatest likelihood for error owing to small samples.

The frequency of locus pairs in apparent disequilibrium ($P < 0.05$) was only slightly higher at hybrid sites (frequency of *marmoratus* alleles between 0.2 and 0.8) than at nonhybrid sites (freqency of *marmoratus* alleles less than 0.2 or greater than 0.8). Apparent linkage disequilibrium at hybrid sites was found for three locus pairs at site 3 in the northern region, for two locus pairs at site 19, and for one pair at site 26 in the central region. Note again, however, that four of these six instances involved the highly polymorphic PEPD and GPI loci. The frequency of 0.33 pairs per hybrid site (6 pairs at 18 possible sites) is scarcely greater than the frequency of 0.27 pairs at nonhybrid sites (7 pairs at 26 possible sites; table 17), especially

considering that the three pairs at hybrid site 3 all involved PEPD or GPI.

Tests were also conducted on six pooled groups (one group for each of the three transects and one group for each of their associated sites). In tests with the pooled genotypic data, only the sMDHP-TF locus pair of the central transect was apparently in linkage disequilibrium. All 84 tests were possible with this pooled evidence. Neither sMDHP-TF nor any other pair of loci was in disequilibrium in the five other pooled groups. Overall, the rarity of locus pairs in apparent disequilibrium suggests that no pairs of the seven highly polymorphic loci are closely linked, and they may be on different chromosomes (table 17).

In conclusion, the small number and scattered distribution of loci in apparent linkage disequilibrium are within the limits of experimental error or drift, suggesting that there is little or no selection against hybrid gene combinations and that mating is random in the hybrid zones. This conclusion is consistent also with the high number of backcross and later generation progeny apparent within the hybrid zones.
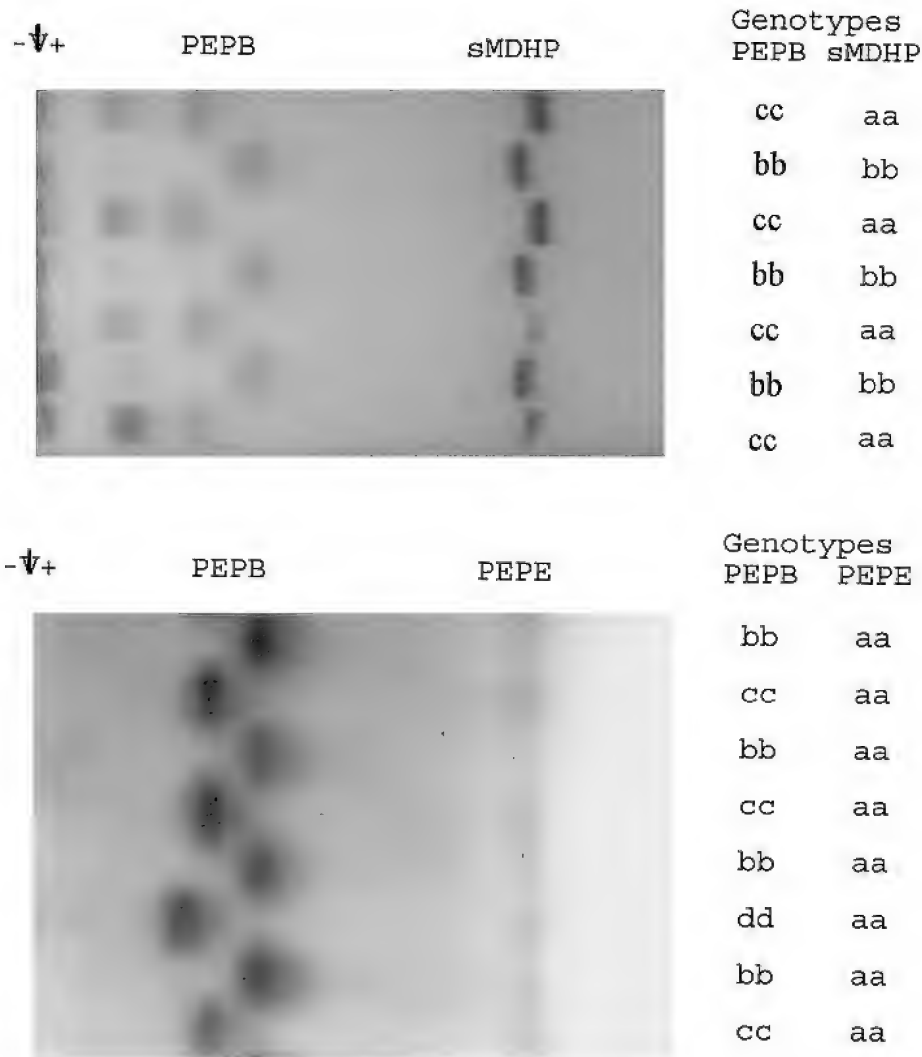
Fig. 28.   Banding patterns of diagnostic loci sMDHP and PEPB. **Top**. Gel stained for sMDHP and then counterstained with substrate for PEPB. The sMDHP a-allele is diagnostic for *marmoratus*, the b-allele for *punctilinealis*. Notice how close the a and b bands are to each other. As sMDHP is a tetrameric protein, the five-banded patterns of heterozygous individuals are often difficult to score with confidence. **Bottom**. Gel stained for PEPB. The b-allele is diagnostic for *punctilinealis*; the c- and d-alleles are diagnostic for *marmoratus*. The substrate for PEPB, leucyl.glycyl.glycine, is also a substrate for PEPE, which is invariant in *C. tigris*. Arrows indicate sites of sample application; anode is to the right.
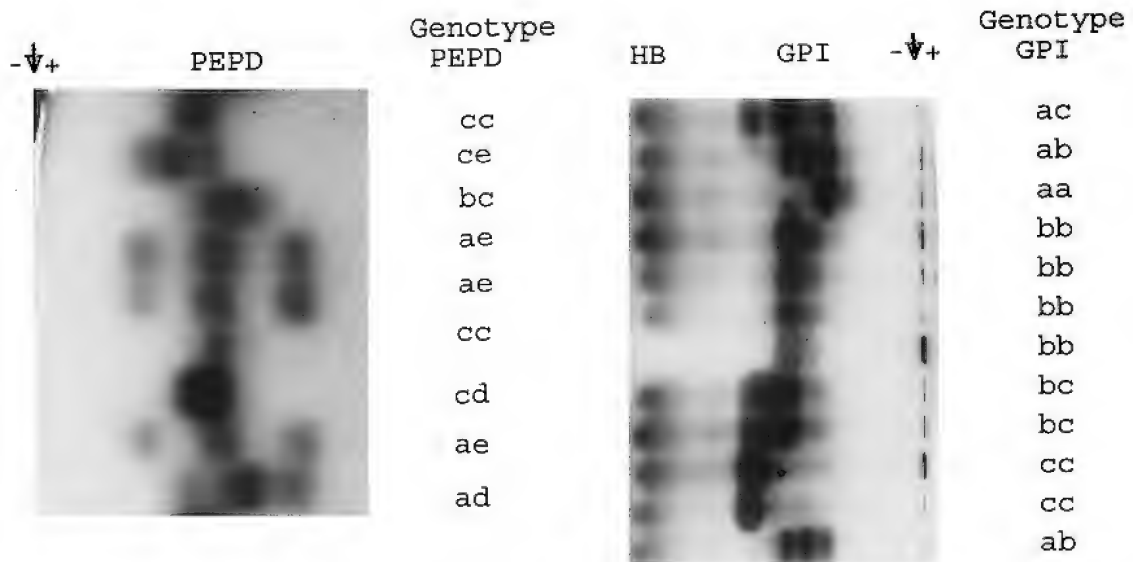
Fig. 29.   Banding patterns illustrating the high polymorphism in PEPD and GPI. **Left**. Gel stained for PEPD, the most polymorphic of the loci examined. The gel shows six combinations of the five PEPD alleles. The a- and b-alleles had the highest frequencies in *punctilinealis*; the c-allele was highest in *marmoratus*. **Right**. Gel stained for GPI. The a- and b-alleles had their highest frequencies in *marmoratus*; the c-allele was most frequent in *punctilinealis*. As this analysis utilized hemolysates, hemoglobin appears as a cathodally migrating band. Arrows indicate sites of sample application; anode is to the right.

HETEROZYGOSITIES

Heterozygosity measurements were useful in evaluating population substructure for lizards from the *Cnemidophorus tigris* contact region. Observed and expected heterozygosities were used to calculate indices of inbreeding at individual sites and for evaluating coancestry across all sites (Hartl and Clark, 1997). Indices of both observed and expected heterozygosities based on the four diagnostic loci (table 15) and on the seven highly polymorphic loci (table 19) were determined for each site consisting of nine or more lizards. As expected, observed values were highest at the center of the hybrid zones where frequencies of *marmoratus* alleles ranged between 0.2 and 0.8.

Contributions of each of the 13 polymorphic loci to heterozygosity at individual sites were measured across each of the three transects (table 18). Loci with low heterozygosities included sIDH, EST1, PEPA, mAAT, sACOH, and PGM3 with values ranging between 0 to 0.233. Values for highly polymorphic PEPD and GPI were most extreme,

ranging between 0.444 to 0.680. Heterozygosities for the diagnostic loci were moderate, ranging from 0.178 to 0.394. The high magnitude of heterozygosity for the seven highly polymorphic loci emphasizes the marked level of gene admixture that characterizes the *C. tigris* hybrid zones, as discussed above.

FIXATION INDICES (F STATISTICS)

Fixation indices were determined to evaluate the substructure of populations in the *Cnemidophorus tigris* contact region. F values for each of the 13 polymorphic loci offered evidence on the contribution of each locus to assessments of inbreeding at individual sites and on the magnitude of genetic differences among subpopulations at different sites (table 18).

The inbreeding coefficient f (F_{is}) is an index of common ancestry between mating pairs in a population, which "measures the fractional reduction in heterozygosity in an inbred population relative to a random-mating subpopulation with the same allele fre-

Fig. 30. Average frequencies of the *marmoratus* alleles at each of the four diagnostic loci and of the *marmoratus* mtDNA haplotype at sites along the northern transect (fig. 4). The frequency change in the IDDH a-allele, which occurs only in *marmoratus*, is also shown. The northern hybrid zone was about 7.8 km wide, with the midpoint of gene exchange at site 3 (compare with fig. 39).

Fig. 31. Average frequencies of the *marmoratus* alleles at each of the four diagnostic loci and of the *marmoratus* mtDNA haplotype at sites along the central transect (fig. 5). The frequency change in the IDDH a-allele, which occurs only in *marmoratus*, is also shown. The central hybrid zone was about 3.2 km wide, with the midpoint of gene exchange at site 26 (compare with fig. 40).
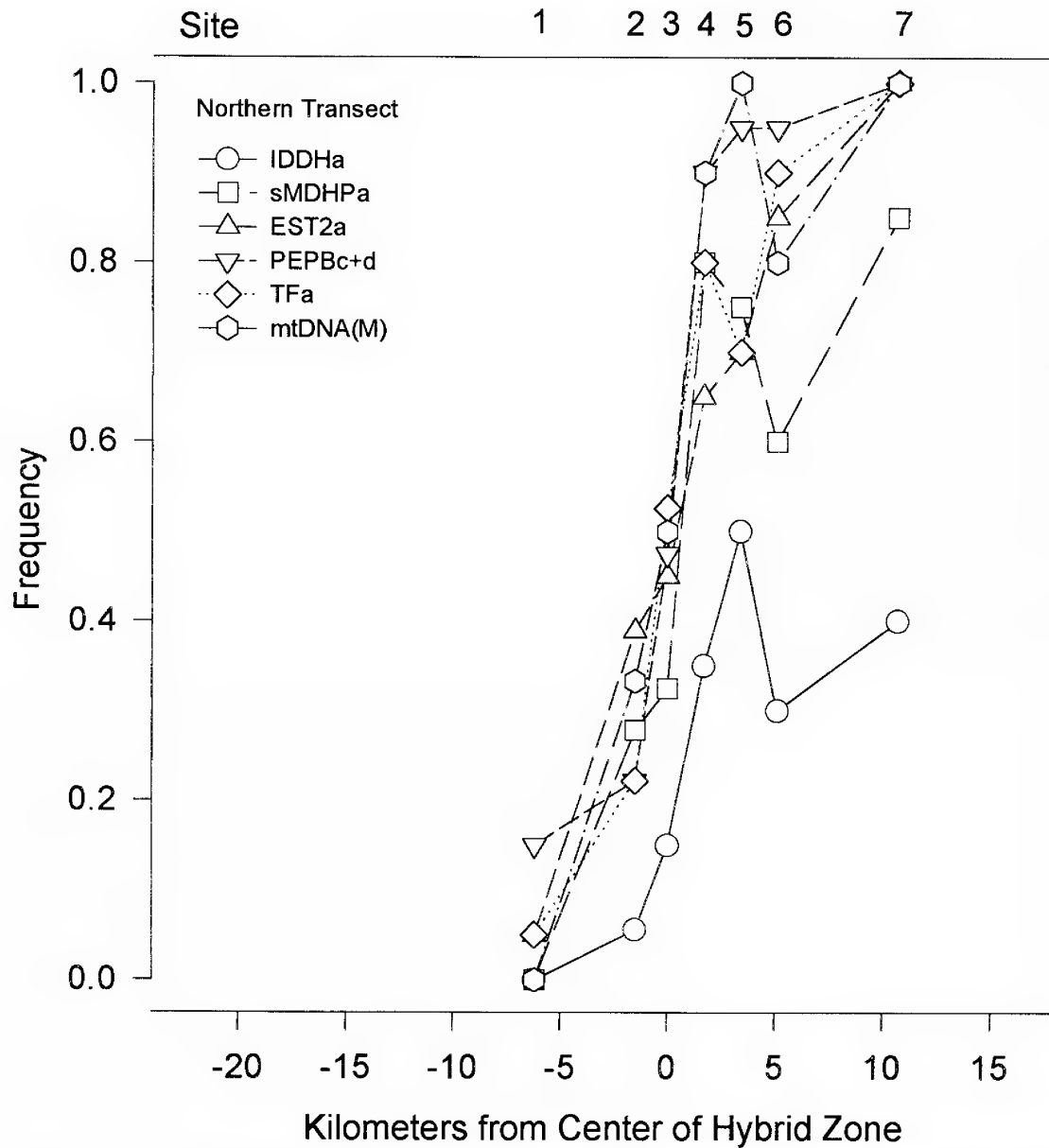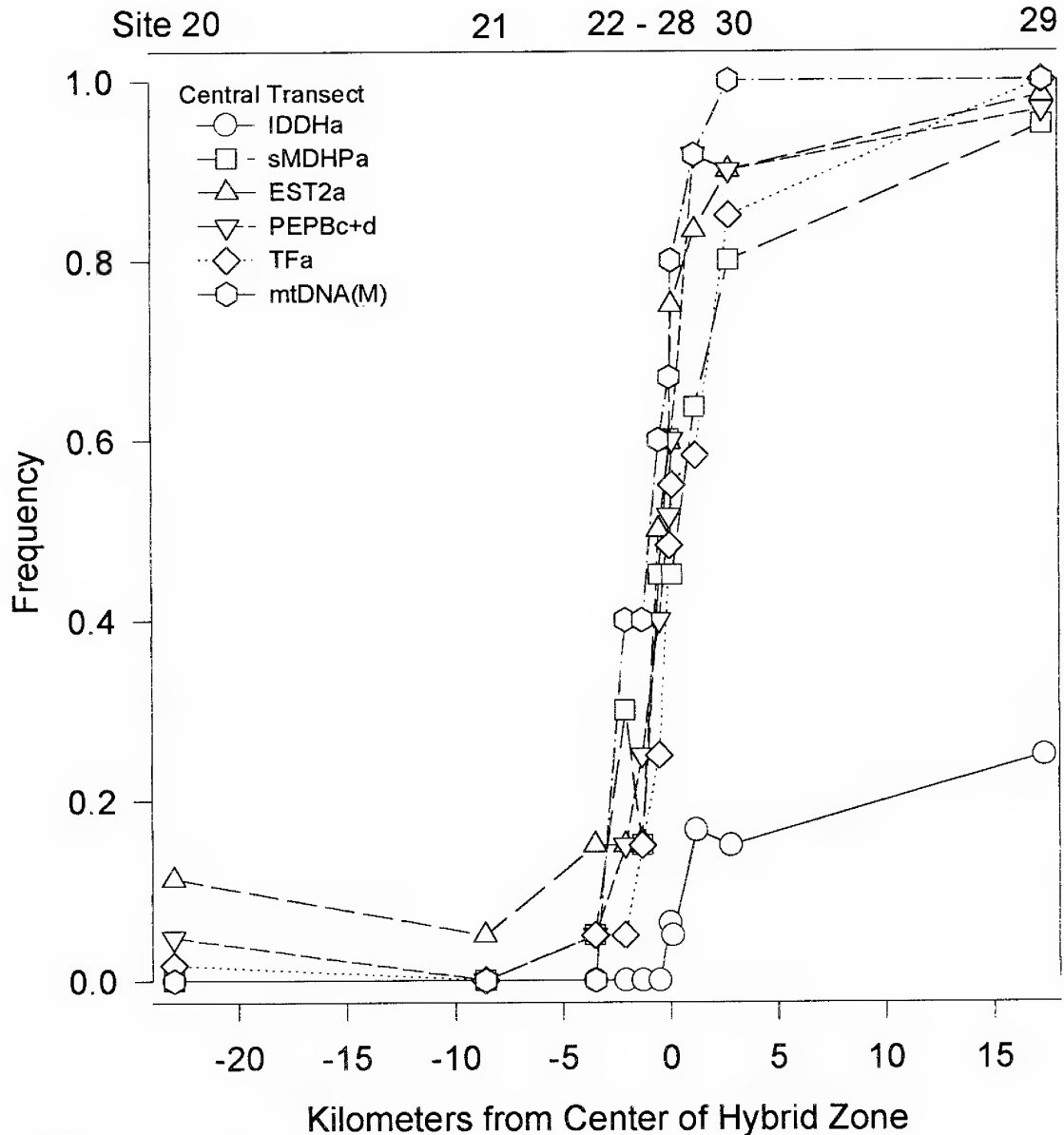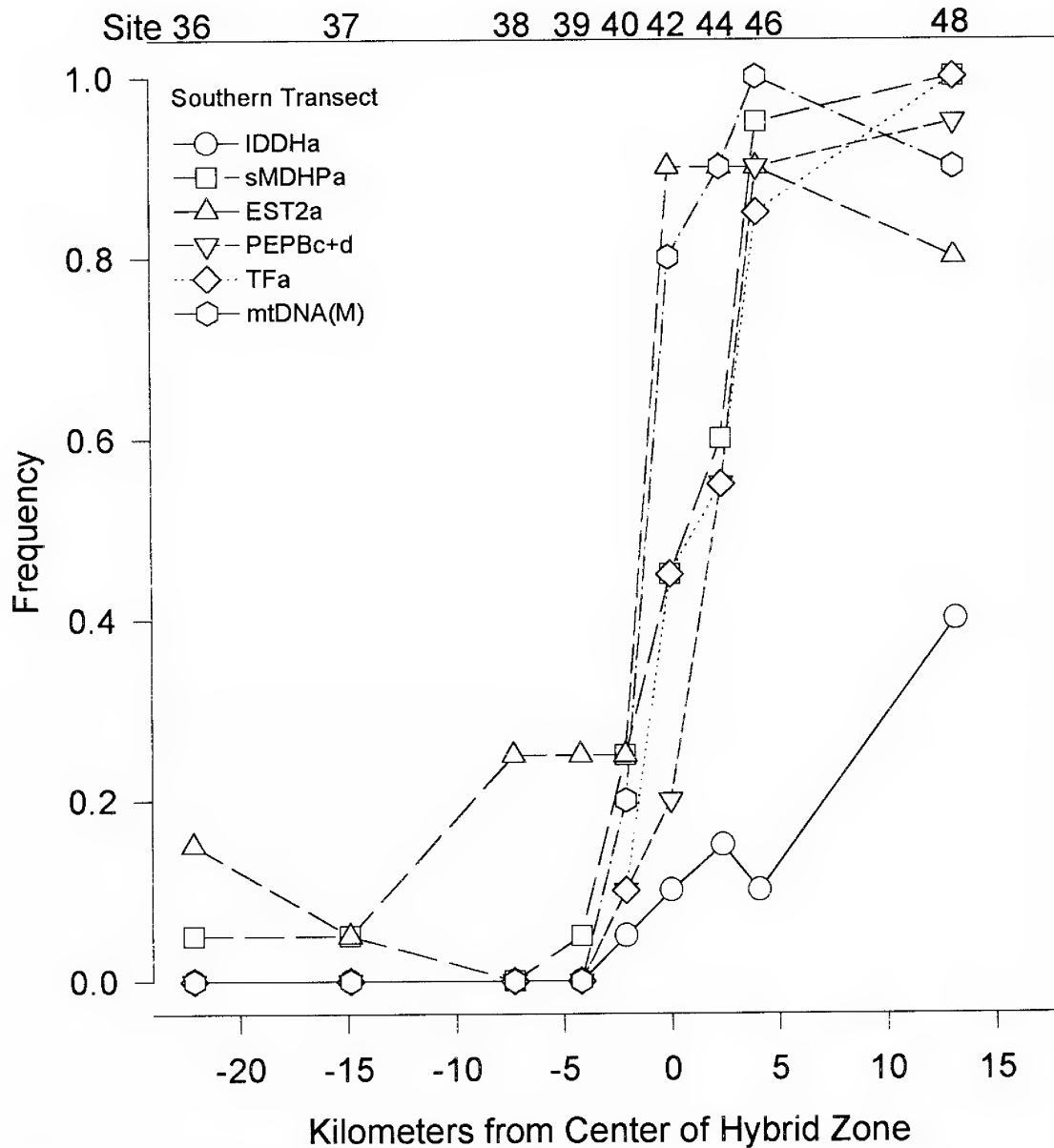
Fig. 32. Average frequencies of the *marmoratus* alleles at each of the four diagnostic loci and of the *marmoratus* mtDNA haplotype at sites along the southern transect (fig. 5). The frequency change in the IDDH a-allele, which occurs only in *marmoratus*, is also shown. The southern hybrid zone was about 5.5 km wide, with the midpoint of gene exchange at site 42 (compare with fig. 41).

TABLE 13
Diversity of Genotypes of the Four Diagnostic Loci among *C. tigris* from Sites Transecting the Hybrid Zones[a]

	Northern Transect Site							Totals
	1	2	**3**	4	5	6	7	
No. lizards	10	9	**20**	10	10	10	10	79
No. genotypes[b]	4	6	**18**	9	7	8	3	38
Pure PUN[c]	6	0	**1**	0	0	0	0	7
F_1 hybrids[d]	0	0	**0**	1	0	0	0	1
F_2+[e]	4	9	**19**	7	9	8	3	59
Pure MAR[f]	0	0	**0**	2	1	2	7	12

	Central Transect Site											Totals
	20	21	22	23	24	25	**26**	27	28	30	29	
No. lizards	31	10	10	10	10	10	**31**	10	12	10	30	174
No. genotypes	5	2	5	7	9	9	**25**	8	9	6	5	34
Pure PUN	24	9	5	2	2	0	**1**	0	0	0	0	43
F_1 hybrids	0	0	0	0	0	0	**1**	1	0	0	0	2
F_2+	7	1	5	8	8	10	**27**	9	11	6	3	95
Pure MAR	0	0	0	0	0	0	**2**	0	1	4	27	34

	Southern Transect Site									Totals
	36	37	38	39	40	**42**	44	46	48	
No. lizards	10	10	10	10	10	**10**	10	10	10	90
No. genotypes	3	3	3	4	7	**9**	9	6	5	35
Pure PUN	6	8	6	6	3	**0**	0	0	0	29
F_1 hybrids	0	0	0	0	1	**0**	1	0	0	2
F_2+	4	2	4	4	6	**10**	8	6	5	49
Pure MAR	0	0	0	0	0	**0**	1	4	5	10

[a] Based on combinations of alleles at the four diagnostic loci. Bold numbers mark the center of each hybrid zone, where the frequency of *marmoratus* alleles is approximately 0.5.

[b] Number of genotypes observed; the maximum possible number of combinations of alleles for the diagnostic loci is 162. Ninety-one different genotypes were observed among the lizards from the three transects.

[c] Number of lizards with a pure *punctilinealis* genotype.

[d] Number of lizards with the heterozygous state at all four diagnostic loci.

[e] Number of lizards with a genotype of an F_2 or higher hybrid or of backcrosses.

[f] Number of lizards with a pure *marmoratus* genotype.

quencies'' (Hartl and Clark, 1997: 137). The contribution of most individual loci to $f(F_{is})$ was very low across most transects. Even the relatively high indices for sIDH of the northern transect and PEPA of the southern transect were not statistically significant (table 18).

Table 19 lists $f(F_{is})$, for lizards from sites across the contact region. Values were calculated from observed and expected heterozygosities at each site. Ninety-five percent confidence limits suggest that there is no sig-nificant deficiency of heterozygotes at most sites. Values of $f(F_{is})$ for only seven of the 45 sites indicated significant heterozygote deficiency, which may be due to the Wahlund effect rather than inbreeding; only one of these sites was from within a hybrid zone transect. The statistical significance of $f(F_{is})$ probably is strongly compromised by the small samples at most sites; the magnitude of $f(F_{is})$ indicated no significant deficiency of heterozygotes at any of the five sites with 20 or more individuals.

TABLE 14
Genetic Differentiation in the Pure Subspecies from the Contact Region

Locus	No. lizards	Allele frequencies					Hetero.[a]	f $(F_{is})^{b}$
		a	b	c	d	e		
				punctilinealis[c]				
IDDH	39	—	1.000	—	—	—	0.000	—
sMDHP	39	0.026	0.974	—	—	—	0.051	−0.013
sIDH	40	0.038	0.962	—	—	—	0.075	−0.026
mAAT	40	—	0.913	0.088	—	—	0.125	+0.229
EST1	40	—	0.925	0.075	—	—	0.150	−0.069
EST2	40	0.075	0.925	—	—	—	0.150	−0.069
PEPA	40	0.013	0.988	—	—	—	0.025	0.000
PEPB	40	—	0.963	0.038	—	—	0.075	−0.026
PEPD	40	0.600	0.275	0.088	0.038	—	0.575	−0.023
sACOH	40	0.050	0.937	0.013	—	—	0.125	−0.043
GPI	40	0.025	0.150	0.825	—	—	0.300	0.000
PGM3	40	0.838	0.163	—	—	—	0.325	−0.182
TF	40	0.013	0.988	—	—	—	0.025	0.000
				marmoratus[d]				
IDDH	199	0.372	0.628	—	—	—	0.434	+0.072
sMDHP	200	0.950	0.050	—	—	—	0.105	−0.053
sIDH	199	0.008	0.992	—	—	—	0.016	−0.005
mAAT	200	—	1.000	—	—	—	0.000	—
EST1	199	0.003	0.987	0.011	—	—	0.026	−0.009
EST2	199	0.942	0.058	—	—	—	0.101	−0.050
PEPA	199	0.002	0.915	0.083	—	—	0.153	+0.043
PEPB	200	—	0.022	0.920	0.058	—	0.135	+0.016
PEPD	199	0.010	0.030	0.837	0.065	0.060	0.291	+0.005
sACOH	199	—	0.990	0.010	—	—	0.021	−0.008
GPI	200	0.420	0.568	0.013	—	—	0.421	+0.161
PGM3	196	0.952	0.041	0.007	—	—	0.081	+0.177
TF	175	0.989	0.011	—	—	—	0.024	−0.009

[a] Observed heterozygosity.

[b] The inbreeding coefficient was estimated with the GENEPOP program of Raymond and Rousset (1995), which uses the method of moments (Weir and Cockerham, 1984).

[c] Includes specimens from sites 1, 21, 36, and 37.

[d] Includes specimens from sites 7, 13–17, 29, 47, 48, and 52–54. Twenty-two of the 27 heterozygotes involved the c-alleles and/or d-alleles at PEPB (both diagnostic alleles for *marmoratus*). Heterozygosity involving *punctilinealis* and *marmoratus* alleles equaled 0.022.

The coancestry coefficient, $\theta(F_{st})$, estimates the magnitude of genetic differences among subpopulations. Coefficients for each of the 13 polymorphic loci were calculated for subpopulations of the three transects. The value of $\theta(F_{st})$ across each transect was highest for the four diagnostic loci and for IDDH, PEPD, and GPI, indicating the value of these seven loci for following gradients of diversity among sites. The values of $\theta(F_{st})$ calculated for individual alleles of PEPD and GPI indicate that alleles that help distinguish the pure subspecies had considerably higher $\theta(F_{st})$s than did other alleles at these loci. The f (F_{it}), the coefficient of inbreeding of an individual, was similar to $\theta(F_{st})$ for most loci at sites along all transects (table 18). This is to be expected when f (F_{is}) equals 0, indicating that random mating prevails in the subpopulations.

In summary, heterozygosity levels for the most variable loci emphasize the high level of gene admixture that is taking place in the *C. tigris* hybrid zones. F statistics suggest that inbreeding is low to absent at most or all sites and that there are extensive genetic

TABLE 15
Indices of Diversity at Sites within the Transects Based on the Four Diagnostic Loci

| Site | No. lizards | Percentage of *marmoratus* alleles | | Heterozygosity[a] | | Genetic distance from *marmoratus* | |
		Proteins[a] (±SE)	mtDNA	Observed (±SE)	Expected (±SE)	Nei[b]	Hillis[c]
				Northern Transect			
1	10	6.3 ± 6.3	0.0	0.123 ± 0.047	0.128 ± 0.053	0.449	0.496
2	9	27.8 ± 7.9	33.3	0.265 ± 0.265	0.261 ± 0.075	0.232	0.268
3	**20**	**44.4 ± 8.5**	**50.0**	**0.244 ± 0.059**	**0.296 ± 0.072**	**0.156**	**0.234**
4	10	78.8 ± 10.3	90.0	0.292 ± 0.072	0.282 ± 0.061	0.029	0.059
5	10	77.5 ± 11.9	100.0	0.269 ± 0.069	0.259 ± 0.062	0.011	0.023
6	10	82.5 ± 15.5	80.0	0.223 ± 0.065	0.238 ± 0.063	0.003	0.014
7	10	96.3 ± 7.5	100.0	0.162 ± 0.054	0.176 ± 0.057	0.000	0.000
				Central Transect			
20	31	4.5 ± 5.0	0.0	0.164 ± 0.052	0.175 ± 0.050	0.517	0.503
21	10	1.3 ± 2.5	0.0	0.146 ± 0.065	0.142 ± 0.060	0.521	0.595
22	10	7.5 ± 5.0	0.0	0.162 ± 0.056	0.157 ± 0.056	0.441	0.480
23	10	16.3 ± 10.3	40.0	0.192 ± 0.066	0.204 ± 0.068	0.341	0.375
24	10	16.3 ± 6.3	40.0	0.208 ± 0.064	0.192 ± 0.050	0.375	0.405
25	10	40.0 ± 10.8	60.0	0.285 ± 0.085	0.289 ± 0.074	0.160	0.186
26	**31**	**54.4 ± 5.4**	**67.7**	**0.311 ± 0.078**	**0.303 ± 0.073**	**0.093**	**0.132**
27	10	58.7 ± 12.5	100.0	0.323 ± 0.079	0.304 ± 0.071	0.065	0.086
28	12	75.1 ± 17.1	91.7	0.214 ± 0.061	0.232 ± 0.068	0.027	0.031
30	10	86.3 ± 4.8	100.0	0.231 ± 0.061	0.202 ± 0.049	0.009	0.041
29	30	97.5 ± 2.1	100.0	0.116 ± 0.037	0.127 ± 0.043	0.000	0.000
				Southern Transect			
36	10	5.0 ± 14.1	0.0	0.200 ± 0.047	0.192 ± 0.044	0.528	0.576
37	10	2.5 ± 2.8	0.0	0.146 ± 0.056	0.126 ± 0.043	0.527	0.546
38	10	6.3 ± 12.6	0.0	0.154 ± 0.056	0.182 ± 0.054	0.486	0.512
39	10	7.5 ± 11.7	0.0	0.200 ± 0.069	0.189 ± 0.062	0.407	0.383
40	10	17.5 ± 8.7	20.0	0.269 ± 0.064	0.255 ± 0.058	0.326	0.326
42	**10**	**50.0 ± 29.1**	**80.0**	**0.254 ± 0.063**	**0.296 ± 0.064**	**0.160**	**0.209**
44	10	65.0 ± 47.1	90.0	0.285 ± 0.068	0.307 ± 0.069	0.069	0.140
46	10	90.0 ± 4.5	100.0	0.169 ± 0.050	0.180 ± 0.054	0.009	0.025
48	10	93.8 ± 5.4	90.0	0.169 ± 0.068	0.161 ± 0.057	0.000	0.000

[a] Percentage of *marmoratus* alleles summed over the diagnostic loci (sMDHP, EST2, PEPB, and TF). Mean number of alleles per locus based on the 13 polymorphic loci ranged from 1.6 to 2.2 and was similar for each transect. Sites 3, 26, and 42 are the 50:50 points of gene exchange (midpoints) of their respective transects.

[b] Unbiased estimates based on the 13 polymorphic loci (BIOSYS-1).

[c] This follows Hillis (1984).

differences among subpopulations (tables 18 and 19).

CYTONUCLEAR DISEQUILIBRIUM TESTS

Analyses were conducted to test whether there are associations between any cytoplasmic genes and nuclear genes or genotypes in specimens of *Cnemidophorus tigris* from the contact region. Random associations would be consistent with an absence of selection and a pattern of random mating (nonassortative) among the lizards. For these analyses

we used the nuclear genes and genotypes of the four diagnostic loci versus the two local haplotypes of 12S ribosomal mtDNA.

Ideally, these analyses should be conducted only with large samples of local demes, particularly as the contingency tables are expected to include six cells or more. Our site samples were not large enough for individual testing, so rather than conduct no tests whatsoever, we decided to pool selected samples and proceed cautiously with interpretations. In addition, for the PEPB locus we treated

TABLE 16

Loci at Which Genotype Frequencies Were Apparently Not in Hardy-Weinberg Equilibrium[a]

Site	Locus	Prob.
3	PEPB	0.012
7	GPI	0.029
12	EST2	0.046
20	EST2	0.028
29	IDDH	0.048
32	GPI	0.046
35	PEPB	0.046
45	PEPD	0.029
46	PEPD	0.028

[a] $P < 0.05$; the seven highly polymorphic loci were tested for sites with at least 10 specimens. Site 3 is at the center of the northern hybrid zone where the frequency of *marmoratus* alleles is approximately 0.5. Hardy-Weinberg Chi-square tests (exact tests) were carried out with the GENEPOP program of Raymond and Rousset (1995). Twenty-two of the 315 tests were not possible, largely because of the absence of polymorphism at some sites of nonhybrids. Global tests on pooled data for each transect showed equilibrium at each locus.

the d-alleles of *C. t. marmoratus* as if they were c-alleles (pooling d with c) to reduce the number of cells involved for testing PEPB.

For use in calculating cytonuclear disequilibria, nuclear and cytoplasmic data from lizards of the three transects and their associated sites were organized into contingency tables for the northern, central, and southern hybrid regions (append. 4). These respective hybrid regions include specifically only those specimens from sites within the step-clines where the frequencies of the *marmoratus* alleles averaged between 0.2 and 0.8 (May et al., 1975). Contingency tables for two additional tests included pooled data from all relevant sites across the entire contact region. One composite group, Entire Region I, included the data pooled from the three hybrid regions that were first tested separately. The second composite group, Entire Region II, was restricted to pooled data from only those sites at the centers of the hybrid regions, where the mean frequency of *marmoratus* alleles averaged from 0.4 to 0.6 (append. 4).

Cytonuclear disequilibria indices thus were calculated separately for geographic subsets in the northern, central, and southern hybrid regions and for the two Entire Region

TABLE 17

Pairs of the Seven Highly Polymorphic Loci Showing Apparent Significant Linkage (Genotypic) Disequilibrium at Individual Sites Throughout the Contact Region[a]

Site[b]	N[c]	Locus pairs[d]	P (±SE)
1	10	PEPD-GPI	0.016 ± 0.002
3	20	sMDHP-PEPD	0.036 ± 0.005
		sMDHP-GPI	0.020 ± 0.003
		PEPD-TF	0.018 ± 0.003
19	10	sMDHP-GPI	0.014 ± 0.002
		PEPB-TF	0.039 ± 0.003
20	31	EST2-PEPD	0.008 ± 0.002
		GPI-TF	0.033 ± 0.002
26	31	sMDHP-TF	0.043 ± 0.003
29	30	IDDH-sMDHP	0.040 ± 0.002
30	10	sMDHP-TF	0.033 ± 0.001
32	10	PEPD-GPI	0.033 ± 0.004
45	10	IDDH-PEPB	0.034 ± 0.002

[a] Probability tests of contingency tables for each pair of loci for population samples at each site in the contact region were performed with the GENEPOP program (Raymond and Rousett, 1995).

[b] Only sites with 10 or more specimens were tested. Sites from the center of a hybrid zone are shown in bold type.

[c] Number of lizards.

[d] Locus pairs in apparent linkage disequilibrium. Of tests on pooled data for each transect and for each set of associated sites, only the combination sMDHP-TF in the central hybrid zone was in apparent disequilibrium ($P < 0.05$).

groups. These indices include gametic phase disequilibrium D (D^A_M), an estimate of the extent of departure from no association between cytoplasmic haplotypes and nuclear alleles, and D_1 (D^{AA}_M), D_2 (D^{Aa}_M), and D_3 (D^{aa}_M), which estimate departures from no association between cytoplasmic haplotypes and each of the three nuclear genotypes (Asmussen et al., 1987; Arnold, 1993), where the subscript M refers to the *marmoratus* mtDNA haplotype and superscripts refer to nuclear alleles. Because of limitations owing to ranges of allele frequencies, each disequilibrium has a maximum range from -0.25 to $+0.25$. To facilitate comparisons and evaluations of disequilibria, indices were normalized to maximum ranges of between -100% and $+100\%$ (Asmussen and Basten, 1996). A normalized index of 0% ($D = 0$) indicates a completely random association (no associ-

TABLE 18
F Statistics[a] and Heterozygosity for the 13 Polymorphic Loci

Locus	f (F_{is})	F (F_{it})	θ (F_{st})[b]	H ($\pm$SE)[c]
		Northern Transect		
IDDH	0.070	0.190	0.129*	0.316 ± 0.064
sMDHP	0.072	0.379	0.331*	0.312 ± 0.058
sIDH	0.395	0.384	−0.017	0.029 ± 0.014
mAAT		Not possible		0.000 ± 0.000
EST1	−0.006	−0.022	−0.016	0.057 ± 0.015
EST2	−0.166	0.220	0.331*	0.394 ± 0.074
PEPA	−0.060	−0.029	0.030	0.079 ± 0.029
PEPB	0.165	0.491	0.390*	0.285 ± 0.028
PEPD[d]	0.061	0.192	0.139*	0.576 ± 0.107
sACOH	−0.065	0.003	0.064	0.029 ± 0.021
GPI[d]	0.114	0.262	0.167*	0.680 ± 0.073
PGM3	−0.041	−0.041	0.000	0.096 ± 0.045
TF	0.123	0.478	0.405*	0.249 ± 0.046
Overall			0.247*	0.218 ± 0.060
		Central Transect		
IDDH	0.161	0.249	0.105*	0.100 ± 0.027
sMDHP	−0.021	0.492	0.502*	0.317 ± 0.062
sIDH	−0.009	−0.019	−0.010	0.045 ± 0.012
mAAT	0.104	0.152	0.054*	0.048 ± 0.048
EST1	0.003	−0.008	−0.011	0.018 ± 0.009
EST2	0.091	0.547	0.502*	0.246 ± 0.037
PEPA	−0.100	−0.045	0.050*	0.097 ± 0.028
PEPB	−0.163	0.431	0.510*	0.331 ± 0.079
PEPD[d]	0.047	0.332	0.299*	0.483 ± 0.060
sACOH	0.141	0.188	0.055*	0.036 ± 0.016
GPI[d]	−0.037	0.111	0.143*	0.589 ± 0.042
PGM3	−0.061	−0.024	0.035*	0.233 ± 0.038
TF	0.073	0.598	0.567*	0.236 ± 0.055
Overall			0.348*	0.214 ± 0.050
		Southern Transect		
IDDH	−0.125	0.061	0.166*	0.016 ± 0.075
sMDHP	0.012	0.604	0.599*	0.189 ± 0.098
sIDH	0.000	−0.026	−0.026	0.056 ± 0.015
mAAT	0.020	0.054	0.035	0.156 ± 0.037
EST1	−0.108	−0.028	0.072*	0.100 ± 0.042
EST2	0.068	0.518	0.483*	0.256 ± 0.026
PEPA	0.372	0.378	0.009	0.067 ± 0.029
PEPB	0.050	0.617	0.597*	0.178 ± 0.088
PEPD[d]	0.037	0.225	0.195*	0.533 ± 0.073
sACOH	−0.059	−0.040	0.018	0.100 ± 0.034
GPI[d]	0.048	0.267	0.230*	0.444 ± 0.065
PGM3	0.016	0.032	0.016	0.233 ± 0.054
TF	−0.142	0.603	0.652*	0.189 ± 0.079
Overall			0.351*	0.194 ± 0.041

[a] Tests were carried out with the GENEPOP program of Raymond and Rousset (1995) in which fixation indices were estimated with the method of moments (Weir and Cockerham, 1984). The indices of *f, F,* and θ are equivalent to Wright's F_{is}, F_{it} and F_{st}, respectively.

[b] θ (F_{st}) indices designated with asterisks were statistically significantly different from a null model of θ (F_{st}) (i.e., no substructure; exact test, $P < 0.05$).

[c] Heterozygosity, direct count.

[d] θ (F_{st}) values were high for individual alleles of the four diagnostic loci, the a-, b-, and c-alleles of PEPD, and the b- and c-alleles of GPI.

TABLE 19

Heterozygosities and Inbreeding Coefficients for Sites in the Contact Region Based on the Seven Highly Polymorphic Loci[a]

Site	Heterozygosity[a] Observed (±SE)	Heterozygosity[a] Expected (±SE)	Inbreeding coefficient[b] f (F_{is})
	Northern Transect		
1	0.200±0.076	0.209±0.087	0.045
2	0.460±0.098	0.455±0.080	−0.013
3	**0.410±0.051**	**0.511±0.051**	**0.197***
4	0.471±0.081	0.455±0.043	−0.038
5	0.457±0.069	0.438±0.046	−0.047
6	0.400±0.065	0.400±0.068	0.000
7	0.243±0.081	0.274±0.085	0.263
	Northern Associate		
9	0.343±0.102	0.314±0.082	0.096
10	0.257±0.141	0.278±0.112	0.080
11	0.300±0.109	0.309±0.103	0.026
12	0.300±0.087	0.398±0.082	0.257*
13	0.257±0.078	0.263±0.083	0.024
14	0.201±0.061	0.222±0.065	0.050
	Central Transect		
20	0.157±0.074	0.182±0.079	0.139
21	0.171±0.113	0.172±0.105	0.005
22	0.243±0.087	0.240±0.087	−0.013
23	0.314±0.101	0.338±0.099	0.073
24	0.300±0.095	0.280±0.067	−0.074
25	0.486±0.108	0.470±0.085	−0.036
26	**0.514±0.079**	**0.497±0.068**	**−0.034**
27	0.500±0.090	0.477±0.071	−0.050
28	0.373±0.060	0.427±0.065	0.084
30	0.371±0.078	0.320±0.057	−0.172
29	0.176±0.059	0.198±0.070	0.112
	Central Associate		
16	0.243±0.107	0.208±0.078	−0.181
18	0.357±0.107	0.450±0.078	0.215*
19	0.529±0.099	0.489±0.086	−0.086
31	0.271±0.052	0.294±0.057	0.081
	Southern Transect		
36	0.157±0.065	0.151±0.062	−0.042
37	0.143±0.095	0.116±0.068	−0.250
38	0.157±0.100	0.190±0.100	0.182
39	0.243±0.117	0.237±0.106	−0.027
40	0.386±0.096	0.365±0.086	−0.061
42	**0.414±0.067**	**0.441±0.077**	**0.058**
44	0.443±0.078	0.473±0.068	0.048
46	0.271±0.061	0.296±0.066	0.088
48	0.300±0.105	0.285±0.081	−0.056
	Southern Associate		
32	0.186±0.046	0.255±0.062	0.282*
33	0.329±0.036	0.410±0.058	0.207*
34	0.329±0.052	0.392±0.071	0.170*
35	0.343±0.100	0.361±0.086	0.053
41	0.343±0.061	0.450±0.062	0.247*

TABLE 19—(*Continued*)

Site	Heterozygosity[a] Observed (±SE)	Heterozygosity[a] Expected (±SE)	Inbreeding coefficient[b] f (F_{is})
43	0.486±0.101	0.420±0.087	−0.106
45	0.357±0.065	0.368±0.060	0.021
47	0.271±0.125	0.291±0.092	−0.204

[a] Determined with the BIOSYS-1 program of Swofford and Selander (1981). Sites with fewer than nine individuals were not included in the calculations. The seven highly polymorphic loci are IDDH, sMDHP, EST2, PEPB, PEPD, GPI, and TF.

[b] Ninety-five percent confidence intervals suggest that a significant deficiency of heterozygotes occurred at sites with values designated with asterisks; however, only one such site is in a transect. Confidence limits for all other sites suggest no significant deficiency; f (F_{is}) values are not significantly different from zero (GDA-version d11 program of Lewis and Zaykin, 1998).

[c] Sites numbered in bold are from centers of hybrid zones where the frequency of *marmoratus* alleles is approximately 0.5.

ation) between nuclear genes and cytoplasmic haplotypes; a normalized index of 100% ($D = -0.25$ or $+0.25$) indicates a completely nonrandom association of nuclear genes and haplotypes.

All D values were very low to moderate (table 20). Although there is significant variation from locus to locus, for many loci the standard errors of the D values are not significantly different from 0.

The lowest D values across all loci for one pooled sample were those for Entire Region II, which includes those sites at the centers of the hybrid regions (table 20; *marmoratus* allele frequencies of 0.4–0.6). The pattern of D values was similar to that of the other regions tested, but all D values were of very low magnitude, generally much closer to 0 than in the other tests (table 20).

As we interpret these D values, we must consider the effects of pooling samples and the possibility of being misled. For example, a rather poor choice of pooling would be to pool samples of only pure *C. t. punctilinealis* and pure *C. t. marmoratus* as if they were from one deme. The results would strongly and incorrectly suggest that these taxa do not interbreed, an erroneous conclusion that would be based on inappropriate pooling of

TABLE 20
Cytonuclear Disequilibria (±SEs) for the Four Nuclear Diagnostic Loci and the Mitochondrial Haplotypes[a]

Locus	No. lizards	D	D_1	D_2	D_3
		Northern Hybrid Region[b]			
sMDHP	49	0.082 ± 0.024	0.093 ± 0.025	-0.023 ± 0.033	-0.070 ± 0.033
EST2	49	0.042 ± 0.024	0.052 ± 0.029	-0.021 ± 0.034	-0.031 ± 0.029
PEPB	49	0.080 ± 0.029	0.068 ± 0.032	0.024 ± 0.028	-0.092 ± 0.032
TF	48	0.022 ± 0.027	0.029 ± 0.033	-0.013 ± 0.034	-0.016 ± 0.031
Normalized (D′, percent)[c]					
sMDHP		50.3	82.2	10.2	32.7
EST2		34.1	46.1	11.4	13.3
PEPB		38.7	27.1	27.9	52.7
TF		15.0	21.7	5.9	9.6
		Central Hybrid Region[b]			
sMDHP	70	0.011 ± 0.019	0.009 ± 0.022	0.004 ± 0.026	-0.012 ± 0.023
EST2	70	0.038 ± 0.016	0.072 ± 0.022	-0.067 ± 0.026	-0.005 ± 0.019
PEPB	70	0.025 ± 0.014	0.048 ± 0.017	-0.047 ± 0.024	-0.001 ± 0.020
TF	69	0.029 ± 0.019	0.024 ± 0.021	0.009 ± 0.026	-0.034 ± 0.025
Normalized (D′, percent)[c]					
sMDHP		8.5	13.4	3.1	6.8
EST2		34.9	71.4	48.4	4.0
PEPB		14.8	50.8	23.5	0.5
TF		18.5	29.6	5.2	14.5
		Southern Hybrid Region[b]			
sMDHP	70	0.074 ± 0.021	0.079 ± 0.025	-0.009 ± 0.032	-0.070 ± 0.028
EST2	69	0.083 ± 0.024	0.079 ± 0.028	0.008 ± 0.027	-0.087 ± 0.026
PEPB	70	0.038 ± 0.022	0.024 ± 0.024	0.030 ± 0.028	-0.053 ± 0.029
TF	70	0.084 ± 0.019	0.074 ± 0.020	0.020 ± 0.029	-0.094 ± 0.027
Normalized (D′, percent)[c]					
sMDHP		54.2	95.4	4.9	36.5
EST2		32.4	40.7	6.3	54.8
PEPB		22.9	24.8	18.7	22.2
TF		53.7	90.2	11.3	40.8
		Entire Region-I[d]			
sMDHP	189	0.043 ± 0.012	0.050 ± 0.013	-0.015 ± 0.017	-0.035 ± 0.017
EST2	188	0.058 ± 0.013	0.069 ± 0.016	-0.022 ± 0.017	-0.047 ± 0.015
PEPB	188	0.050 ± 0.013	0.046 ± 0.015	0.007 ± 0.017	-0.053 ± 0.016
TF	187	0.055 ± 0.013	0.050 ± 0.014	0.009 ± 0.017	-0.060 ± 0.017
Normalized (D′, percent)[c]					
sMDHP		28.3	60.7	7.5	16.4
EST2		41.1	49.8	10.2	32.9
PEPB		33.6	44.0	4.9	27.8
TF		35.4	50.1	6.6	28.5
		Entire Region-II[e]			
sMDHP	100	0.033 ± 0.016	0.029 ± 0.016	0.007 ± 0.023	-0.036 ± 0.022
EST2	100	0.038 ± 0.016	0.063 ± 0.021	-0.051 ± 0.023	-0.012 ± 0.018
PEPB	100	0.009 ± 0.016	0.007 ± 0.018	0.006 ± 0.024	-0.012 ± 0.022
TF	98	0.013 ± 0.017	0.011 ± 0.019	0.004 ± 0.023	-0.015 ± 0.022
Normalized (D′, percent)[c]					
sMDHP		24.1	46.0	4.8	19.0
EST2		32.2	17.1	29.8	10.7
PEPB		7.2	12.3	4.1	4.8
TF		12.6	15.5	12.7	18.3

samples. Entire Region II is our pooled sample ($N = 100$) that comes closest to approximating a sample of a deme in the center of a transect, and it shows the lowest D values across the board (table 20). When examining all 16 D values, locus by locus, for Entire Region II, we find that the 95% confidence interval for 13 of them clearly overlaps the value of 0. The three exceptions are for EST2, and one of these is close to 0 (0.038 ± 0.016).

We conclude that the nuclear genes and genotypes are randomly associated with the cytoplasmic haplotypes in the hybrid zones. This suggests that hybridization has been both extensive and of long duration, as the associations of cytoplasmic and nuclear alleles found in the original pure parental forms have been disassociated in the hybrid zones.

Goodness-of-fit of observed D values was tested against values predicted by each of five hypotheses about possible mating patterns in a hybrid zone (G tests, table 21; Asmussen et al., 1987). Various alternative hypotheses are discussed in table 21. The hypothesis with the greatest support is that with the lowest G value (highest P). We focus on the results for Entire Region II, as this pooled sample comes closest to approximating a large sample from one deme in the center of a transect, and we review the results one locus at a time. For sMDHP, the hardest locus to score because of the similar mobilities on the gels (fig. 28), H2 has the highest P (best fit), while HR, H1, and H3 are also tenable ($P > 0.05$). For EST2, H3 is the only tenable hypothesis. For PEPB, HR has the best fit, although all five hypotheses are tenable. For TF, H2 has the best fit, but HR is close to it, although all five hypotheses are tenable again.

The hypothesis of random mating in an old hybrid zone (HR) is tenable for three of the four loci; it is the best fit for PEPB, and a close second for TF. This does not constitute strong grounds for rejecting the random mating hypothesis, although there were three different best-fit hypotheses for the four loci. The ambiguities in our results may well reflect the pooling of the samples, drift, and violations of certain assumptions in the models. For example, these populations of *Cnemidophorus* are not closed; they include gene flow, which involves some influx of pure parental forms into the hybrid zones. Also, *C. tigris* has overlapping generations. Nevertheless, for Entire Region II we are impressed by the low D values for most loci (table 20), with most of them at about 0, which is consistent with random mating.

UNCOMMON ALLELES

Electrophoretic variants of low frequency occur at many loci in population samples of *Cnemidophorus tigris* (fig. 33). Such alleles in other species have been used to estimate mutation rates (see Harris et al., 1974; Harris, 1975) and to study gene flow (Slatkin, 1981, 1985a, 1985b, 1987; Barton et al., 1983; Barton and Slatkin, 1986; Slatkin and Barton, 1989).

The term "rare allele" is often used to describe variants of low frequency. Unfortunately, the term has different meanings for different investigators. In their comprehensive evaluation of uncommon variants in hu-

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←

[a] Calculated with the DFIT Fortran programs (Asmussen et al., 1987) using the contingency tables in appendix 4. For PEPB, frequency of the d-allele was added to the frequency of the c-allele (considered equivalent for purposes of these calculations, owing to very small sample sizes).

[b] Data included are for sites with mean frequencies of *marmoratus* alleles between 0.2 and 0.8. Data were pooled for each respective transect and its associated sites.

[c] Maximum disequilibria modified to overcome limitations owing to gene frequencies (see Asmussen and Basten, 1996).

[d] This includes sites with mean frequencies of *marmoratus* alleles between 0.2 and 0.8 pooled over the entire contact region.

[e] This includes sites with mean frequencies of *marmoratus* alleles between 0.4 and 0.6 pooled over the entire contact region (sites 3, 19, 25–27, 42, and 44).

TABLE 21
_G_ Tests[a] of Cytonuclear Disequilibria (table 20) for Goodness-of-Fit to Indices Expected under Various Alternative Mating Patterns[a]

Locus	Northern region		Central region		Southern region		Entire region-I[b]		Entire region-II[c]	
	G	P	G	P	G	P	G	P	G	P
					Hypothesis H1					
sMDHP	10.06	0.002	1.51	0.697	5.96	0.015	12.40	0.000	2.71	0.100
EST2	2.83	0.092	9.20	0.002	7.35	0.007	17.33	0.000	8.31	0.004
PEPB	4.08	0.043	5.69	0.017	0.95	0.035	8.97	0.003	0.12	0.727
TF	0.75	0.387	1.18	0.278	11.57	0.001	11.57	0.001	0.30	0.586
					Hypothesis H2					
sMDHP	0.48	0.489	0.21	0.886	1.63	0.202	0.77	0.380	0.08	0.772
EST2	0.38	0.540	7.09	0.008	0.09	0.771	1.67	0.197	5.04	0.025
PEPB	0.68	0.408	3.60	0.058	1.09	0.296	0.16	0.690	0.05	0.817
TF	0.15	0.698	1.29	0.720	0.63	0.428	0.30	0.581	0.03	0.865
					Hypothesis H3					
sMDHP	4.78	0.029	0.31	0.577	0.32	0.572	4.67	0.031	2.98	0.085
EST2	1.25	0.263	0.06	0.803	10.54	0.001	10.32	0.001	0.49	0.482
PEPB	9.08	0.003	0.00	0.950	3.28	0.070	11.03	0.001	0.31	0.577
TF	0.27	0.604	2.00	0.157	10.71	0.001	13.16	0.000	0.47	0.492
					Hypothesis HD					
sMDHP	10.28	0.001	0.33	0.567	2.81	0.094	11.70	0.001	4.30	0.038
EST2	3.05	0.081	—[d]	—	—	—	19.72	0.000	—	—
PEPB	7.54	0.006	—	—	2.77	0.096	13.95	0.000	0.32	0.570
TF	0.69	0.407	2.22	0.137	16.21	0.000	17.53	0.000	0.56	0.453
					Hypothesis HR					
sMDHP	10.82	0.004	0.36	0.837	12.09	0.002	13.19	0.001	4.31	0.116
EST2	3.17	0.205	9.78	0.008	11.79	0.003	19.96	0.000	8.40	0.015
PEPB	9.15	0.010	5.94	0.051	3.30	0.192	14.15	0.001	0.35	0.841
TF	0.77	0.680	2.35	0.309	16.23	0.000	17.63	0.000	0.58	0.749

[a] Maximum likelihood tests are estimates with different cytonuclear disequilibria set to 0 (Asmussen et al., 1987). Hypothesis H1 sets $D_1 = 0$, $D = D_2/2 = -D_3/2$, and $D \neq 0$. This suggests directionality to pure parental matings with hybrids preferentially backcrossing to the less discriminating parent. Hypothesis H2 sets $D_2 = 0$, $D = D_1 = -D_3$, and $D \neq 0$. This suggests that both taxa mate assortatively and there is no directionality to hybrid matings. Hypothesis H3 sets $D_3 = 0$, $D = D_1/2 = -D_2/2$, and $D \neq 0$. This suggests directionality to pure parental matings with hybrids preferentially backcrossing to the less discriminating parent. Hypothesis HD sets $D = 0$, $D_1 = D_3 = -D_2/2$, and $D_1 \neq 0$. This suggests that nuclear allele frequencies are identical in the two cytotypes and that there is mixed mating. Hypothesis HR sets $D = D_1 = D_2 = D_3 = 0$. This suggests random mating in a fairly old hybrid zone. Best-fit hypotheses have the lowest G and highest P values. A listing of sites for which diagnostic allele frequencies were used to calculate the D values is given in the contingency tables (appendix 4).

[b] This includes sites with mean _marmoratus_ allele frequencies between 0.2 and 0.8 for the four diagnostic loci (see appendix 4).

[c] This includes sites with mean _marmoratus_ allele frequencies between 0.4 and 0.6 for the four diagnostic loci (see appendix 4).

[d] Blanks are from calculations where the computer program became stuck in an infinite loop or tried to take the square root of a negative number.

mans, Harris et al. (1974) define rare alleles as electrophoretic variants that are so uncommon that they probably represent recent mutations. To Slatkin and colleagues, rare alleles may have much higher frequencies and are often present in a number of different populations. In some literature (Raymond and Rousset, 1995), the less specific term "private allele" (Cavalli-Sforza and Bodmer, 1971; Neel, 1973) is used to designate such relatively more common variants that are found in many populations.

To avoid confusion, we follow Harris (1975: 302) and Hartl and Clark (1997) in
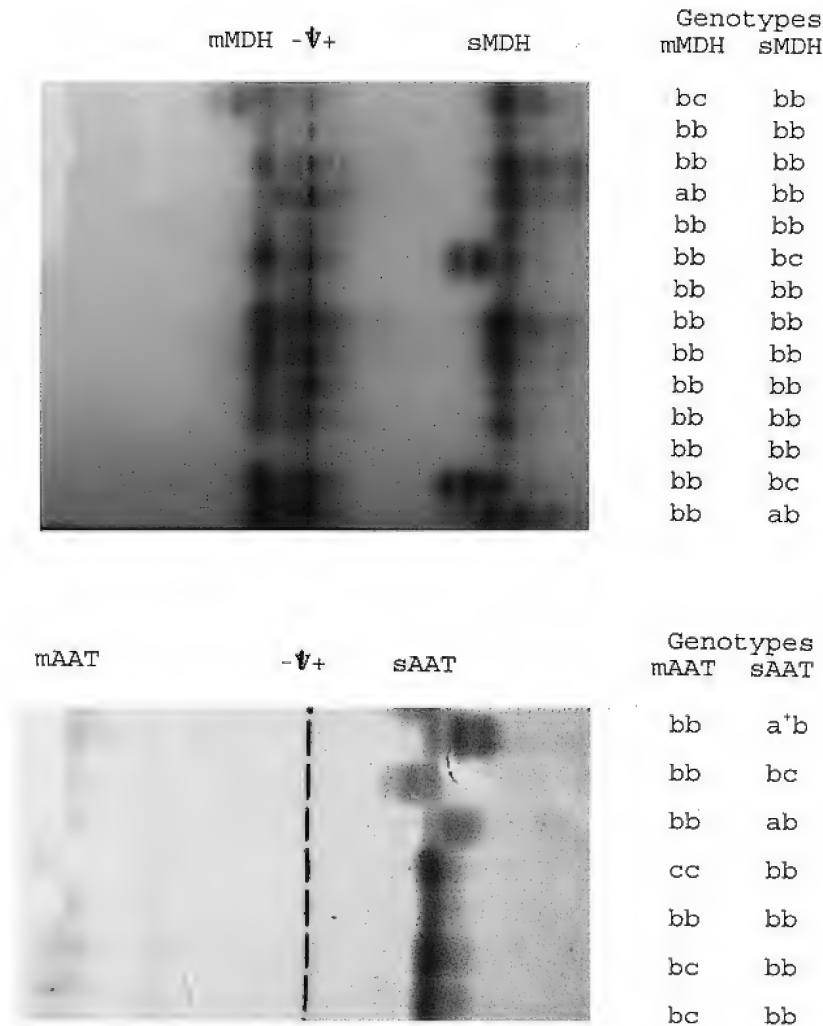
Fig. 33. Gels illustrating uncommon electrophoretic variants in specimens of *C. tigris*. **Top**. Uncommon polymorphisms observed at sMDH and mMDH. **Bottom**. Uncommon polymorphisms observed at sAAT and mAAT. All four enzymes are dimeric, as indicated by the three-banded patterns characteristic of heterozygous individuals. The ab genotype at the sMDH locus was observed in only one specimen of *C. t. aethiops*; the bc genotype at the mMDH locus was observed in only one specimen of *C. t. marmoratus* from Dona Ana County, New Mexico, distant from the contact region. The a+b genotype of sAAT was observed in only one specimen of *punctilinealis* from site 49, distant from the contact region. Arrows indicate sites of sample application; anode is to the right.

defining rare alleles as electrophoretic variants that are "less than 1 in 200 in the general population," so rare that they probably are new mutants. Frequencies of truly rare alleles determined by electrophoretic surveys of huge samples offer important evidence on mutation rates at specific structural gene loci, but Harris (1975) has shown that exception-

ally large samples are required to elucidate mutation rates.

To examine whether specimens of *Cnemidophorus tigris* can shed light on mutation rates or gene flow, we have assembled data on electrophoretic variants of low frequencies found among the 614 lizards from the contact region plus sites 50 and 51 nearby to

TABLE 22
Electrophoretic Alleles of Low Frequency in 614 *Cnemidophorus tigris* from the Contact Region[a]

Locus	Allele	Frequency	Site(s)	Specimen(s)
		Rare Alleles (Frequency of <0.005)		
G3PDH	b	0.0016	14, 39	2
LDH2	a	0.0008	22	1
sMDH	c	0.0016	21, 26	2
mMDH	a	0.0008	48	1
mIDH	b	0.0008	48	1
sAAT	a	0.0041	20, 26, 43	5
sAAT	c	0.0008	14	1
CK1	a	0.0016	13, 14	2
EST1	a	0.0041	12, 29, 38, 39	5
EST2	c	0.0008	33	1
ESTD	a	0.0032	16, 18, 20, 28	4
PEPA	a	0.0041	16, 21, 38,[b] 42	4
PEPB	a	0.0008	11	1
ADA	c	0.0008	22	1
PGM1	b	0.0008	14	1
PGM2	a	0.0008	14	1
PGM3	c	0.0041	2, 13, 14, 20	5
		Other Uncommon Alleles (frequency of >0.005 but <0.02)		
sIDH	a	0.0138	6,[b] 14, 16, 19, 20, 21, 22, 25, 26, 30, 36, 37, 39, 40, 41, 44	17
mAAT	a	0.0065	8, 11, 12, 20,[b] 38	7
EST1	c	0.0146	1, 5, 6, 7, 14, 20, 21, 29, 34, 36, 44, 45	18
ADA	a	0.0081	10, 14, 16, 19, 20,[b] 26	9
sACOH	a	0.0187	4, 9, 11, 20,[b] 25, 34, 36, 37, 38, 40, 42	22
sACOH	c	0.0065	14, 15, 20, 24, 31, 37	8
MPI	a	0.0073	4, 5, 14, 20, 29,[b] 50	8

[a] Sites in the contact region are mapped in figure 3 and the genotypes of individual lizards are given in appendix 3. We include here the specimens from sites 50 and 51 also (appendix 3), because Antelope Pass in the Peloncillo Mountains and the vicinity of Portal, Arizona, are sites close to, although south of, the contact region.

[b] Includes one homozygous genotype.

the south (append. 3). In table 22 these data are assembled in two categories: (1) rare alleles, with average frequencies of less than 0.005 in the total sample; and (2) other uncommon alleles, with frequencies between 0.005 and 0.02 in the total sample. One locus of the rare allele group and four of the other uncommon allele group include lizards with homozygous genotypes; at seven loci of the latter group, the allele was found at between 5 and 16 different sites. It is probable that the a-alleles of sAAT, EST1, ESTD, and PEPA, as well as the c-allele of PGM3 (listed with the rare allele group because of their low frequencies), should be listed with the other uncommon alleles, since they were found at three or four sites, and PEPA of one lizard had a homozygous aa-genotype.

Even though our sample is too small to obtain definitive evidence on frequencies of mutation, the data offer an indication of the rarity of mutation at certain loci. The nine alleles observed only once have the highest chance of giving insight on frequencies of mutation. Of these, there is electrophoretic evidence from additional specimens of *Cnemidophorus tigris* at the LDH2, mMDH, and mIDH loci. Genotypes of none of our 40 specimens from sites distant from the contact region (table 12) nor our 34 specimens of other subspecies (table 31) exhibited these alleles, nor did the 69 *C. tigris* examined by

Parker and Selander (1976). Thus, the a-alleles of LDH2 and mMDH and the b-allele of mIDH have been found only one time each in a total of about 900 lizards. The frequency of these rare alleles is 0.00055.

Some authors have suggested that uncommon electrophoretic variants occur more frequently in hybrid zones than elsewhere. They hypothesize that allelic polymorphism breeds additional polymorphism through the origin of novel, mutant alleles, especially within hybrid zones. Barton and Hewitt (1985) found that in 19 of 23 studies the claim was made for an increase in uncommon variants in hybrid zones. The genetic evidence on *Cnemidophorus tigris* from the contact region offers an opportunity to examine this phenomenon.

If novel alleles are generated in the contact zones, we would expect to find more of them in the center of a hybrid zone than among nonhybrid lizards. Such variants should be more frequent at sites near or at midpoints of gene exchange where heterozygosities are higher than at nonhybrid sites. In the following discussion, the incidence of rare alleles and other uncommon alleles (table 22) at hybrid sites (sites with frequencies of the *marmoratus* alleles at the four diagnostic loci between 0.2 to 0.8) will be compared to the incidence at nonhybrid sites. One might predict that uncommon variants are more frequent at hybrid sites. We found quite the opposite in a variety of tests of the data in table 22.

Using the 14 hybrid and 36 nonhybrid sites (append. 3 and 4) to obtain a summary of the incidence of alleles as presented in table 22, we found electrophoretic variants of low frequency at 20 of the 36 loci. Such variants were present in genomes of lizards from 11 hybrid sites (79%) and from 30 nonhybrid sites (83%). The rare allele group of variants included seven alleles (1.9% of 378 alleles) in lizards from five of the 14 hybrid sites (35.7%) and 32 alleles (3.8% of 856 alleles) in lizards from 11 of the 36 nonhybrid sites (38.9%). The other uncommon allele group included 15 alleles (4.0% of 378) from eight hybrid sites (57.1%) and 72 alleles (8.5% of 856 alleles) from 26 nonhybrid sites (72.2%). No allele was found exclusively at a hybrid

site, although several rare alleles were found exclusively at nonhybrid sites (table 22).

To allow for possible sample size bias (experimental error), we repeated the above analyses using only those samples with 30 or more specimens per site. For nonhybrids, this included sites 20 (*punctilinealis*) and 14 and 29 (*marmoratus*); for hybrids, this included site 26. For the rare allele group, we found 0.08 rare alleles per nonhybrid and 0.06 per hybrid. For the other uncommon allele group, we found 0.12 alleles per nonhybrid and 0.06 per hybrid, or twice as many uncommon alleles in nonhybrids as in hybrids. The two sites with the highest absolute numbers of uncommon alleles (site 14 comprised of 88 *marmoratus* and site 20 comprised of 31 *punctilinealis*) were samples of nonhybrids. These exceeded the number from site 26 at the midpoint of gene exchange in the central transect with a sample of 31 lizards.

Finally, we also examined the occurrence and distribution of only the rarest of the rare alleles to see whether they exist primarily in zones of highest heterozygosities. Among the 614 lizards surveyed for table 22, three-fourths of the rare alleles were found only once or twice, each time in the heterozygous state, for a frequency of either 0.0008 or 0.0016. There were 15 such occurrences, eight at nonhybrid sites (5 from site 14, the largest sample of all) and only one from a hybrid site (site 26 with the largest sample of hybrids). The remaining six were distributed as follows: (1) four from sites of essentially pure *punctilinealis* (sites 21, 22, and 39), (2) one from site 13 of nearly pure *marmoratus*, and (3) one from site 33 of essentially pure *marmoratus* but adjacent to the southern hybrid zone. One might argue that the four rare alleles at sites 22, 23, and 39 should be counted as occurring within a hybrid zone, as they are geographically close and the relevant zones might be moving eastward (see below, "Comparisons With the Past and Predictions for the Future"), and thus a few generations ago sites 22 and 39, in particular, may have been more involved as sites of hybridization. Even so, this would mean that eight of the rarest alleles were found at nonhybrid sites and five at hybrid sites. This also does not constitute support

for the hypothesis concerning the origin and distribution of novel alleles in hybrid zones.

In our preliminary report (Dessauer and Cole, 1991) we noted one inconsistency with this hypothesis. The uncommon variants in *C. tigris* from the central hybrid zone usually occurred at loci for which the nonhybrids were fixed or nearly fixed for the same allele at opposite ends of the transect. This pattern continues to hold true. For 20 of the loci at which one or more rare alleles were found (table 22), 18 are loci at which *punctilinealis* and *marmoratus* have the same allele essentially fixed. The two exceptions, EST2 and PEPB, are diagnostic loci at which the non-hybrids differ significantly. At EST2 only one rare allele was found in a heterozygous individual from site 33 (at the edge of the southern hybrid zone); at PEPB only one rare allele was found in a heterozygous individual from site 11, a nonhybrid sample. For the other five loci at which nonhybrids differ significantly in allele frequencies (IDDH, s-MDHP, PEPD, GPI, and TF), no rare alleles were found in the total sample.

We conclude that there is no relationship between the process of hybridization and the occurrence of rare alleles in *C. tigris* in this contact zone. Even though 19 of 23 electrophoretic surveys reported an increased frequency of novel alleles in hybrid zones (Barton and Hewitt, 1985), our data do not fit this pattern. We urge those who think that there are more rare alleles in hybrid zones to carefully allow for the fact that scientists tend to collect larger samples within hybrid zones than outside of them, and the discovery of uncommon alleles is a function of sample size (Harris et al., 1974; Harris, 1975).

GENE FLOW AND AGE OF THE HYBRID ZONES

It is possible to estimate the age of a hybrid zone using gene-flow data and the width of the step-cline, assuming random mating and neutral (equal fitness among genotypes) secondary contact (Endler, 1977: 93). Considering that we have evidence for extremely little or no selection (none detected) and for random mating, and because we know the widths of the hybrid zones (all discussed above; width of each zone can be measured

on figs. 39–41), all we need is a measure of gene flow to estimate ages.

The standard deviation of the distance moved by an animal during its lifetime is an acceptable gene-flow parameter (Barrowclough, personal commun.; J. C. Hafner et al., 1983; M. S. Hafner et al., 1998); this is sigma or the *l* of some authors. Use of the square root of 2 × sigma (1.41 × sigma) is most appropriate "if dispersal were strictly limited to one dimension" (Rockwell and Barrowclough, 1987: 233), as one might envision as whiptail lizards enter Steins Pass and other passes in the Peloncillo Mountains, or as they follow favorable habitat within drainage systems in the northern part of Animas Valley just south of the Gila River.

There are no published data on the distances moved by individuals of *C. t. punctilinealis* or *C. t. marmoratus* during their lifetime. However, Andrew H. Price used several grids of pitfall traps to gather relevant data on *C. t. marmoratus* in southern New Mexico (see Price, 1986; Price et al., 1993), and he generously sent us his unpublished data on movements. Similarly, Philip C. Rosen used pitfall traps to collect movement data on *C. t. punctilinealis* in southern Arizona, and he also generously sent us a summary of his unpublished data.

The information provided by Price included capture–recapture data on many individuals within measured grids. We selected from his data those lizards for which recaptures spanned a period of at least one year, and for each of the 49 individual *marmoratus* (34 males, 15 females), we measured the straight-line distance between those two points that were farthest apart among all of the capture points. The mean for these 49 lizards was 93.6 m (sigma [standard deviation] of 35.2 m [range = 33 to 217 m]). Therefore, the gene-flow parameter equalled 49.6 m (square root of 2 × sigma, or 1.41 × 35.2). The data provided by Rosen for *punctilinealis* were similar. In addition, he provided observations for 10 hatchlings recaptured over a period of a week to several months, which indicated that they do not disperse widely shortly after hatching.

The number of generations (T) for which the two subspecies of *C. tigris* might have been interbreeding in the three hybrid zones

TABLE 23
Ages of the Hybrid Zones

Transect	w^a (km) (0.2–0.8)	$T_{0.05}{}^b$ (gener-ations)	$T_{0.10}{}^c$ (gener-ations)
Northern	7.8	8500	2100
Central	3.2	1400	360
Southern	5.5	4200	1100

[a] Width of the hybrid zone within the step-cline where frequency of the *marmoratus* alleles varies from 0.2 to 0.8 for the four diagnostic loci (figs. 39–41).

[b] Following Endler (1977:93), $T = 0.35 (w/\sigma)^2$. Instead of σ we used $\sqrt{2}\ \sigma$ (or 1.41σ) as recommended by Rockwell and Barrowclough (1987:233) for situations where dispersal may be "strictly limited to one dimension." For this calculation, $1.41\sigma = 0.05$ km.

[c] What if σ is underestimated by 100%? For this calculation, $1.41\sigma = 0.1$ km. Our field and laboratory observations suggest that generation time in *C. tigris* is 2 years, so age of the hybrid zones in years is $T \times 2$.

under conditions of neutral secondary contact are presented in table 23. The first column of T ($T_{0.05}$) uses the gene-flow parameter 0.05 km. Considering the data used, we are confident that this does not overestimate the gene-flow parameter, but what is the effect if it is a significant underestimate? The second column of T ($T_{0.10}$) uses a gene-flow parameter of 0.10 km, or twice that of the first calculation.

Our field and laboratory observations on *C. tigris* over the years suggest that generation time is two years in this species, so the number of years of interbreeding is $T \times 2$. The central hybrid zone appears to be the youngest of the three, having an age of about 700–2800 years. This age is consistent with expectations based on paleoecological data from packrat middens (see "Comparisons With the Past and Predictions for the Future" below).

The northern hybrid zone appears to be the oldest, aged about 4200–17,000 years, but this estimate may be erroneously long. There are two most likely sources of error, both involving estimation of the width of the hybrid zones. First, we measured the widths from figures 39–41, using only the protein data averaged for the four diagnostic loci and frequencies of the *marmoratus* alleles from 0.2 to 0.8. For the northern hybrid zone, the cutoff point is arguable between sites 4 and 6, and we used the wider figure. In contrast, other summaries of the data show the three hybrid zones to be more similar in width (figs. 42 and 43), suggesting that they are more similar in age.

Second, the way in which we transected the hybrid zones could have produced artifacts. There is no question that the central transect is the clearest, going straight through Steins Pass. The northern transect is perhaps less likely to be a straight line directly across the hybrid zone. Consider the position of this hybrid zone as seen in figure 49. Our transect of sites 1–7 followed the highway and involved collecting in dry creek beds with optimal habitat, but this might not represent the shortest way to transect the hybrid zone as much as it does the route most feasible for efficient collecting. Note that the subspecies appear to contact around site 8, but this was not readily studied because we obtained only two individuals at site 8 despite many attempts to collect there.

In summary, the central transect probably gives the most reliable estimate of width and age of the hybrid zone, in this case, from 700 to 2800 years, and this agrees extremely well with the paleoecological data discussed below.

CYTOGENETICS: KARYOTYPES

The diploid karyotype of *Cnemidophorus tigris* has been described and illustrated previously (Lowe and Wright, 1966; Cole et al., 1969; Lowe et al., 1970b; Cole, 1979; Cole et al., 1988). Basically, it consists of 46 chromosomes, with three pairs of large Set I macrochromosomes (biarmed), 12 pairs of small Set III microchromosomes, and eight pairs of intermediate-size Set II macrochromosomes. All of the subspecies that have been examined have the same karyotype.

In Set I, the largest two pairs are of metacentric chromosomes. The second largest pair bears a dot like satellite distal to the sec-

ondary constriction (the nucleolar organizer region; Ward and Cole, 1986) in a near-terminal position on one arm. The third largest pair is of submetacentric sex chromosomes, which are heteromorphic in males, homomorphic in females. The X and Y chromosomes have a subtle difference in position of the centromere (primary constriction; Cole et al., 1969; Bull, 1978). The Set II chromosomes are all submetacentric, and the Set III microchromosomes are too small for clear resolution of their individual shapes, although many appear biarmed in the clearest preparations.

This karyotype is known to characterize both *C. t. punctilinealis* and *C. t. marmoratus* as well as the following additional subspecies of *C. tigris* that have been examined (Lowe et al., 1970b): *aethiops, estebanensis* (but see Walker et al. [1966] regarding nomenclature, and Wright, [1993]), *maximus*, and *septentrionalis*. Consequently, it has

been our focus in this study to examine karyotypes of *C. t. punctilinealis* × *C. t. marmoratus* hybrids from the contact region for evidence of abnormalities that might, if found, be a consequence of hybridization. We used previously published methods to prepare and study standard, Giemsa-stained chromosomes (Cole, 1979).

We examined 112 somatic cells from 11 lizards (9 males, 2 females) from hybrid site 26 (10 specimens) and hybrid site 41. Site 26 is in the center of the central hybrid zone (fig. 31). The specimens examined were AMNH R-128367 and R-131420–131429.

All of these specimens had normal karyotypes in every detail. In addition, we examined 54 cells from eight specimens (one *punctilinealis*, seven *marmoratus*) from outside the contact region and all were typical for the species as well. All karyotypic data are consistent with there being no breeding or fitness problems among lizards within the hybrid zones.

MORPHOLOGY, REPRODUCTION, AND FITNESS IN THE CONTACT REGION

METHODS

Following Dessauer and Cole (1991), we analyzed six characters of color and pattern (coloration) that distinguish *C. t. punctilinealis* from *C. t. marmoratus*, but which blend together in the hybrid zones. These characters were recorded prior to preservation in a manner to produce a hybrid index, the pure *punctilinealis* condition being scored as 0, the pure *marmoratus* condition as 1.0, intermediates as 0.5, and 0.25 or 0.75 scored for intermediates with strong tendencies toward *punctilinealis* or *marmoratus*, respectively. The coloration hybrid index for an individual is the mean of all six characters, and the score for the site is the mean of those means for the population sample (table 24). The specimens examined and complete locality data are presented in appendix 1; individual hybrid index scores are in appendix 3.

Body size (snout–vent length) and 19 characters of scutellation were recorded for 252 lizards. The characters are described and

discussed by Lowe et al. (1970a), Cole et al. (1988), and Cole and Dessauer (1993). In counting gulars, however, the anteriormost single one that sometimes is present in a central, unpaired condition was ignored, if present, as the counts were also being used for analyses of left side versus right side symmetry. Supralabials and infralabials were counted from the anteriormost one posteriad to the one directly below the posterior edge of the eye (with the eyelids considered as open).

The size and scutellation characters are quite similar in *C. t. punctilinealis* and *C. t. marmoratus*, unlike their coloration. Consequently, we determined and analyzed the full suite of these characters for specimens from selected sites that represent the extremes of the variation and intermediates, as follows: the northern hybrid zone (sites 1, 3, and 7), the central hybrid zone (sites 20, 26, and 29), and the southern hybrid zone (sites 36, 42, and 48). Consequently, for each of the three transects we compared pure *punctilinealis*, pure *marmoratus*, and hybrids from the mid-

point of gene exchange (see "Biochemical Genetics" above). In addition, we recorded the full suite of data for specimens of *marmoratus* from site 14, our largest population sample ($N = 88$), as a guide for understanding local variation, including sexual dimorphism and symmetry.

Margaret G. Arnold analyzed the morphological data with the STATGRAPHICS Plus program (version 6.0), licensed from Manugistics, Inc. Univariate data reductions and *t*-tests were used to analyze sexual dimorphism (unpaired comparisons) and bilateral symmetry (paired comparisons). Principal components analyses (PCAs) were also used, as each character could not be assumed to represent a completely independent estimate of the patterns of variation among these lizards. Nine characters were used for the PCAs of morphology. These characters included snout–vent length and the following scale counts: circumorbitals, infralabials (to posterior corner of eye), gulars (ignoring unpaired central anteriormost one if present), granules around midbody, femoral pores, interfemoral scales, fourth toe lamellae, and fourth finger lamellae. Owing to some missing characters on one side or the other for paired characters (e.g., toe lamellae), the number of specimens analyzed was maximized by using counts from only one side (the right side, but we substituted the left side for rare individuals missing the right). PCAs were performed on the correlation matrices using only specimens with complete data; no estimates for missing values were used. The analyses include variation among and within populations, as several population samples were involved in each PCA.

Sex, enlargement of testes, and estimates of egg-clutch sizes were determined by dissection. Clutch size was obtained by counting the total number (left side plus right side) of either conspicuously enlarging (yolking) ovarian oocytes or shelled, oviductal eggs.

RESULTS

EXTERNAL CHARACTERS

COLORATION AND COMPARISON WITH BIOCHEMICAL GENETICS: In terms of color and pattern, a typical individual of *C. t. punctilinealis* (hybrid index of 0) has the following characters regardless of sex (figs. 34 and 35): (1) four to six distinct dorsal light stripes on body (but fading with age); (2) dorsal light spots on body distinct and usually in the dark fields; (3) light spots (not bars) on sides of body; (4) throat black or brown, perhaps with a red tint; (5) chest black; and (6) posterior lateral abdominal scales white.

In contrast, a typical individual of *C. t. marmoratus* (hybrid index of 1.0) has the following (figs. 34 and 35): (1) dorsal stripes generally not complete or clear, reduced in number if present; (2) dorsal spots continuous with lateral bars and/or coalescing; (3) vertical bars on sides of body; (4) throat white or orange with scattered black spots; (5) chest orange with some scales less than half black; and (6) posterior lateral abdominal scales tan or yellowish tan.

Hybrids (figs. 34 and 35) have intermediate characters or a blend of the characters of both pure taxa, and they exhibit an extraordinary range of variation. Table 24 presents the coloration data for lizards from the 48 sites in the contact region, including the three transects, and figures 36–38 illustrate variation across the transects. Note that the observed ranges of data and standard errors of the means are considerably greater for samples of hybrids than for either pure taxon, consistent both with expectations for hybrid zones with free interbreeding and with the genetic data (see "Biochemical Genetics" above). In fact, in the center of each transect (sites 3, 26, and 42) the range of variation includes literally or nearly all of the full spectrum available, whereas variation is much less at the ends of the transects (figs. 36–38). Also note that variation is less in pure samples of *C. t. punctilinealis* than in pure *C. t. marmoratus* (table 24; figs. 36–38), indicating that our characterization of pure *marmoratus* is not as reliable as that of pure *punctilinealis*. Nevertheless, there is remarkable consistency and agreement among the coloration characters observed in the separate transects of the three hybrid zones and the accompanying genetic data.

Figures 39–41 summarize the means of the protein data for the four diagnostic loci (sMDHP a-allele, EST2a, PEPBc+d, and TFa), mtDNA(M), and the coloration hybrid index for the three transects. This univariate
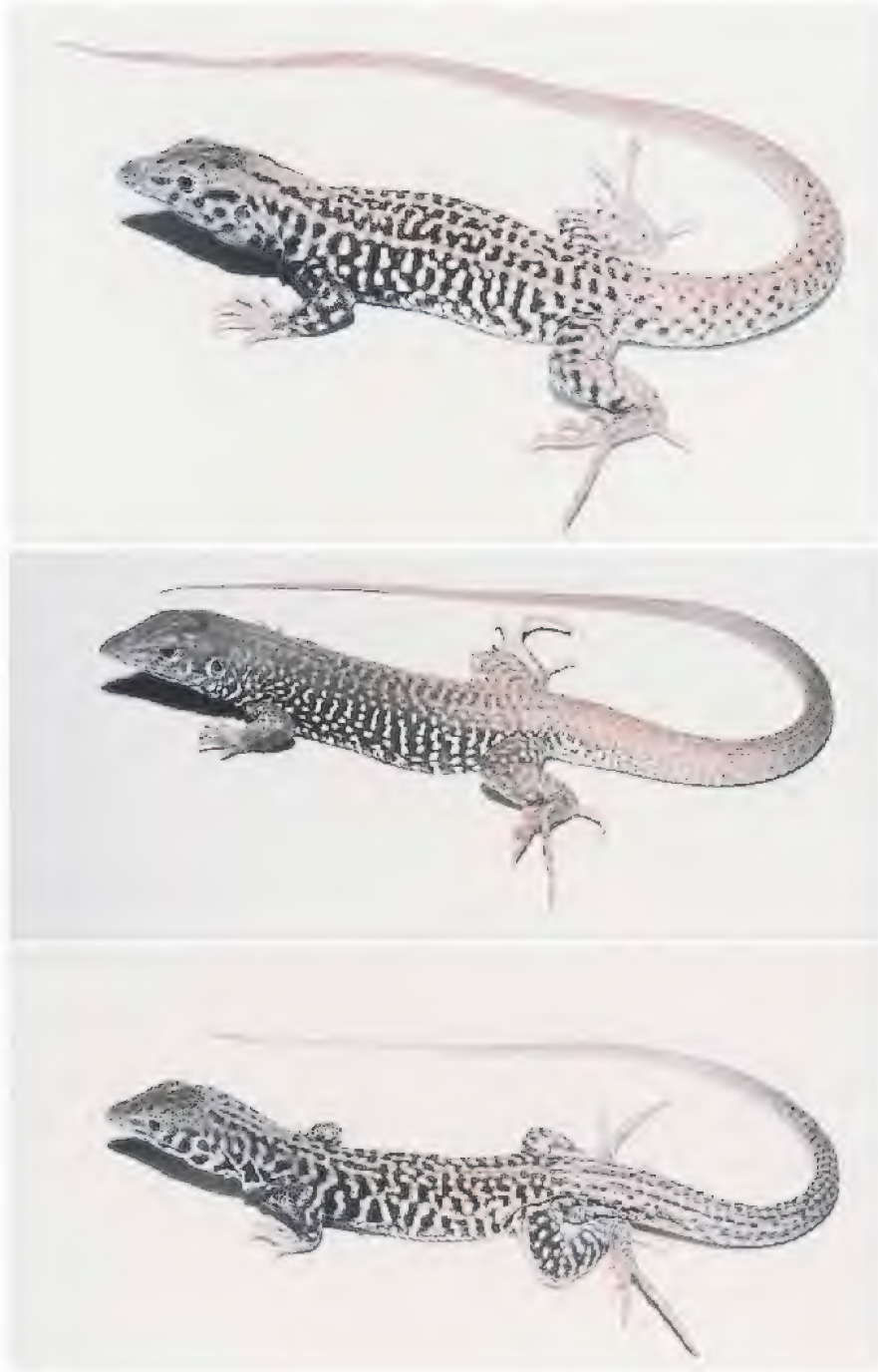
Fig. 34.   Western whiptail lizards (*Cnemidophorus tigris*) from the contact region. **Top**. Pure *C. t. punctilinealis* male, AMNH R-139694, from site 20, body length 87 mm. **Middle**. Hybrid *C. t. punctilinealis* × *C. t. marmoratus*, male, AMNH R-139719, from site 3, body length 96 mm. **Bottom**. Pure *C. t. marmoratus* male, AMNH R-139711, from site 29, body length 77 mm.
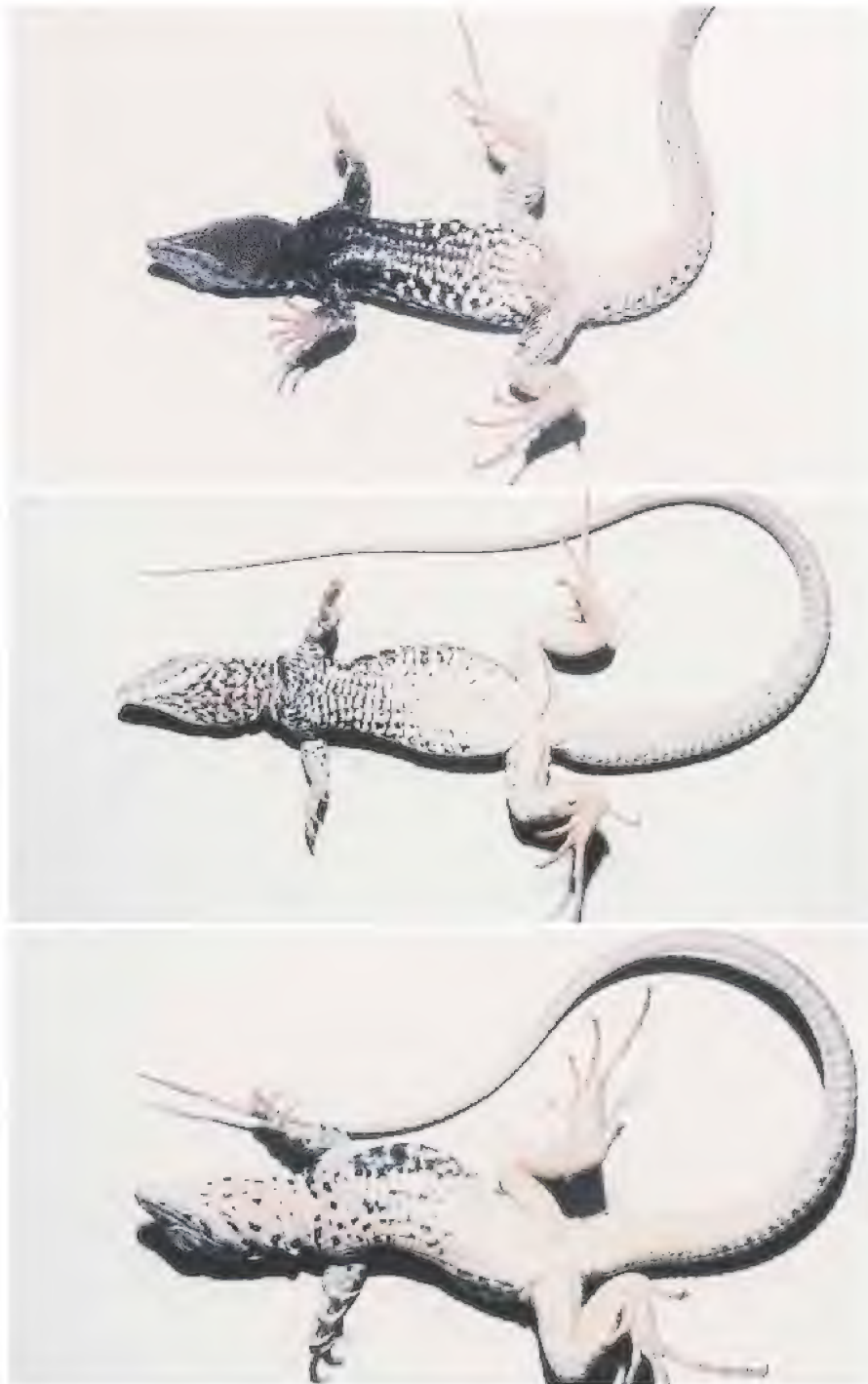
Fig. 35. Ventral views of the same lizards arranged in the same sequence as in figure 34.

TABLE 24

Coloration Hybrid Indices for Lizards from the Contact Region[a]

Site	Hybrid index[b]	Ident.[c]
	Northern Transect	
1	0.02±0.009 (0.0–0.08) 10	PUN
2	0.06±0.028 (0.0–0.21) 9	HYB
3	**0.37±0.052 (0.0–1.0) 20**	**HYB**
4	0.61±0.062 (0.33–0.88) 10	HYB
5	0.78±0.049 (0.58–1.0) 10	HYB
6	0.86±0.031 (0.71–1.0) 10	MAR
7	0.88±0.041 (0.54–1.0) 10	MAR
	Northern Associated Sites	
8	0.44 (0.04–0.83) 2	HYB
9	0.21±0.060 (0.0–0.67) 10	PUN
10	0.04±0.025 (0.0–0.25) 10	PUN
11	0.10±0.037 (0.0–0.33) 10	PUN
12	0.16±0.091 (0.0–0.92) 10	PUN
13	0.92±0.029 (0.71–1.0) 10	MAR
14	0.89 (0.83–0.92) 3	MAR
15	—	MAR
	Central Transect	
20	0.00±0.004 (0.0–0.12) 32	PUN
21	0.00±0.000 (0.0–0.0) 10	PUN
22	0.04±0.019 (0.0–0.17) 10	PUN
23	0.17±0.061 (0.0–0.50) 10	PUN
24	0.22±0.046 (0.0–0.42) 10	PUN
25	0.34±0.057 (0.17–0.75) 10	HYB
26	**0.48±0.052 (0.04–1.0) 31**	**HYB**
27	0.52±0.049 (0.25–0.75) 10	HYB
28	0.80±0.033 (0.67–1.0) 10	HYB
30	0.87±0.044 (0.58–1.0) 10	MAR
29	0.92±0.015 (0.62–1.0) 31	MAR
	Central Associated Sites	
16	0.96±0.012 (0.88–1.0) 10	MAR
17	1.00 1	MAR
18	0.78±0.068 (0.33–1.0) 10	HYB
19	0.36±0.077 (0.04–0.75) 10	HYB
31	0.92±0.029 (0.75–1.0) 10	MAR
	Southern Transect	
36	0.00±0.000 (0.0–0.0) 10	PUN
37	0.01±0.008 (0.0–0.08) 10	PUN
38	0.01±0.006 (0.0–0.04) 10	PUN
39	0.04±0.014 (0.0–0.12) 10	PUN
40	0.19±0.065 (0.0–0.5) 9	PUN
42	**0.46±0.128 (0.04–1.0) 8**	**HYB**
44	0.56±0.063 (0.25–1.0) 10	HYB
46	0.85±0.041 (0.67–1.0) 10	MAR
48	0.92±0.028 (0.75–1.0) 10	MAR
	Southern Associated Sites	
32	0.81±0.040 (0.67–1.0) 10	MAR
33	0.74±0.055 (0.46–1.0) 10	HYB
34	0.07±0.028 (0.0–0.25) 10	PUN
35	0.03±0.018 (0.0–0.17) 10	PUN
41	0.30±0.060 (0.0–0.58) 10	HYB

TABLE 24—(*Continued*)

Site	Hybrid index[b]	Ident.[c]
43	0.16±0.052 (0.0–0.5) 10	PUN
45	0.74±0.045 (0.5–1.0) 10	HYB
47	0.94±0.018 (0.83–1.0) 10	MAR

[a] Figure 3 maps the contact region and collecting sites; figures 4 and 5 map the three transects. Appendix 1 presents complete locality data and specimens examined. Sites 3, 26, and 42 are the 50:50 points of gene exchange (midpoints) of their respective transects.

[b] Mean ± standard error of the mean (observed range) sample size.

[c] Identification: HYB = hybrid; MAR = *C. t. marmoratus*; PUN = *C. t. punctilinealis,* based on percentage of *marmoratus* alleles at the four diagnostic loci being 0.0–19.9 (PUN), 20.0–79.9 (HYB), or 80.0–100.0 (MAR), as in table 15.

analytical approach indicates that the clines for the diverse types of data (morphology, nuclear genes, mitochondrial DNA) are concordant and coincident. In addition, the clines for the three transects (northern, central, and southern) are of basically similar size and shape (figs. 39–41). Similar results were obtained with a multivariate approach.

Principal components analyses (PCAs) were performed on the six coloration characters that show significant geographic variation across the transects of the three hybrid zones (fig. 42). Similarly, PCAs were also performed on the seven most variable gene loci across the same transects (fig. 43). For each analysis, we used the sample means for the frequency (percent) of the *C. t. marmoratus* characters present. We used the arcsine square-root transformation of the mean frequencies to reduce the correlation of variance of frequency with the mean (Sokal and Rohlf, 1981). Principal components were extracted from the variance–covariance matrix to detect patterns common to all characters involved (Barrowclough and Johnson, 1988), as in our preliminary report (Dessauer and Cole, 1991). Finally, the scores for principal component 1 (PC1) for each transect were plotted against sample locality in the transect as distance from the center of the hybrid zone (midpoint of gene exchange, or 50:50 allele frequencies; figs. 42 and 43). For these two plots (mean coloration indices and mean
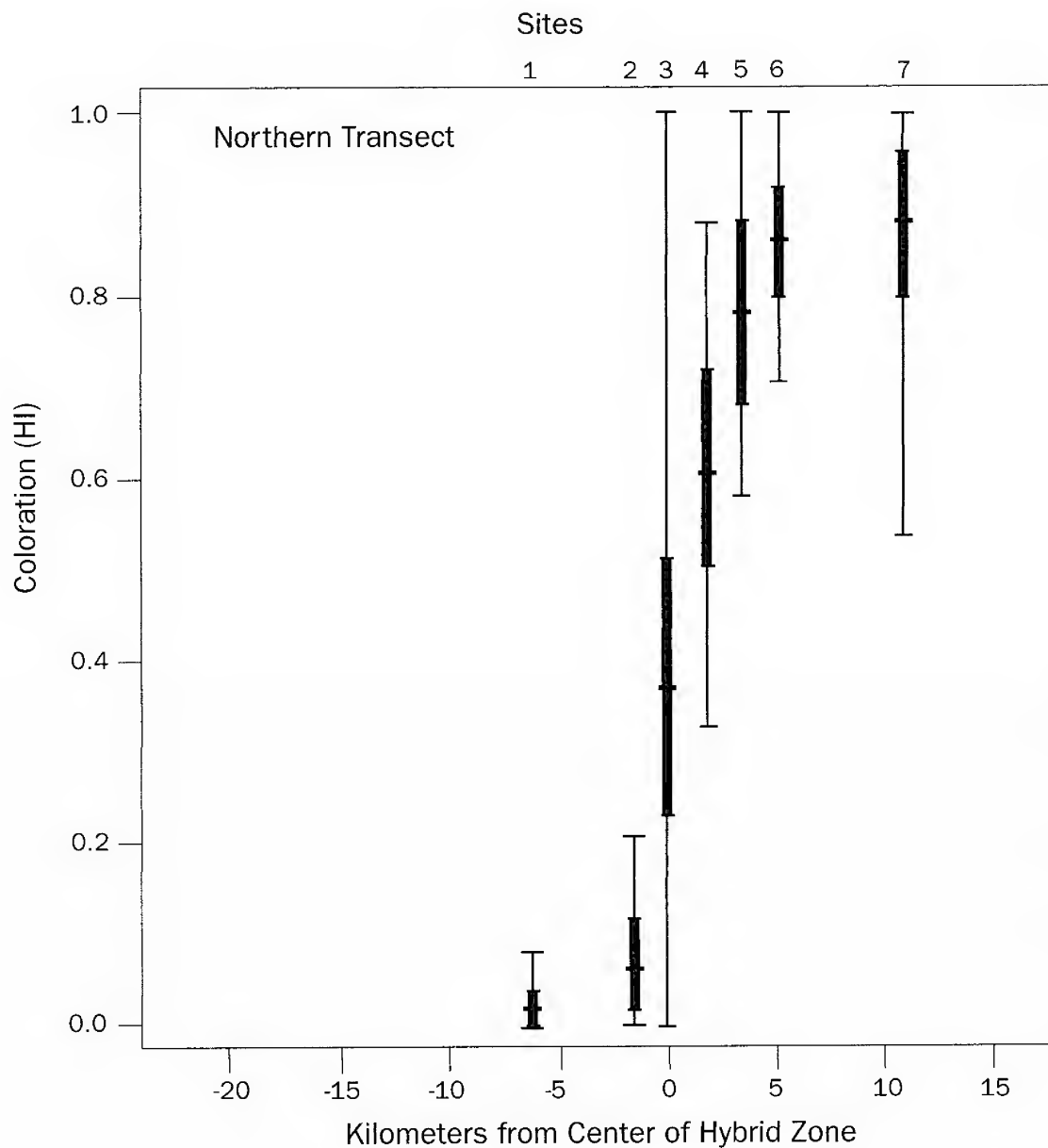
Fig. 36. Frequencies of the *marmoratus* body coloration characters at sites along the northern transect (fig. 4). Site 3 was the center of the northern hybrid zone in genetic characters (fig. 30). Vertical line represents range of data, thick bar the mean, black rectangle the 95% confidence interval.
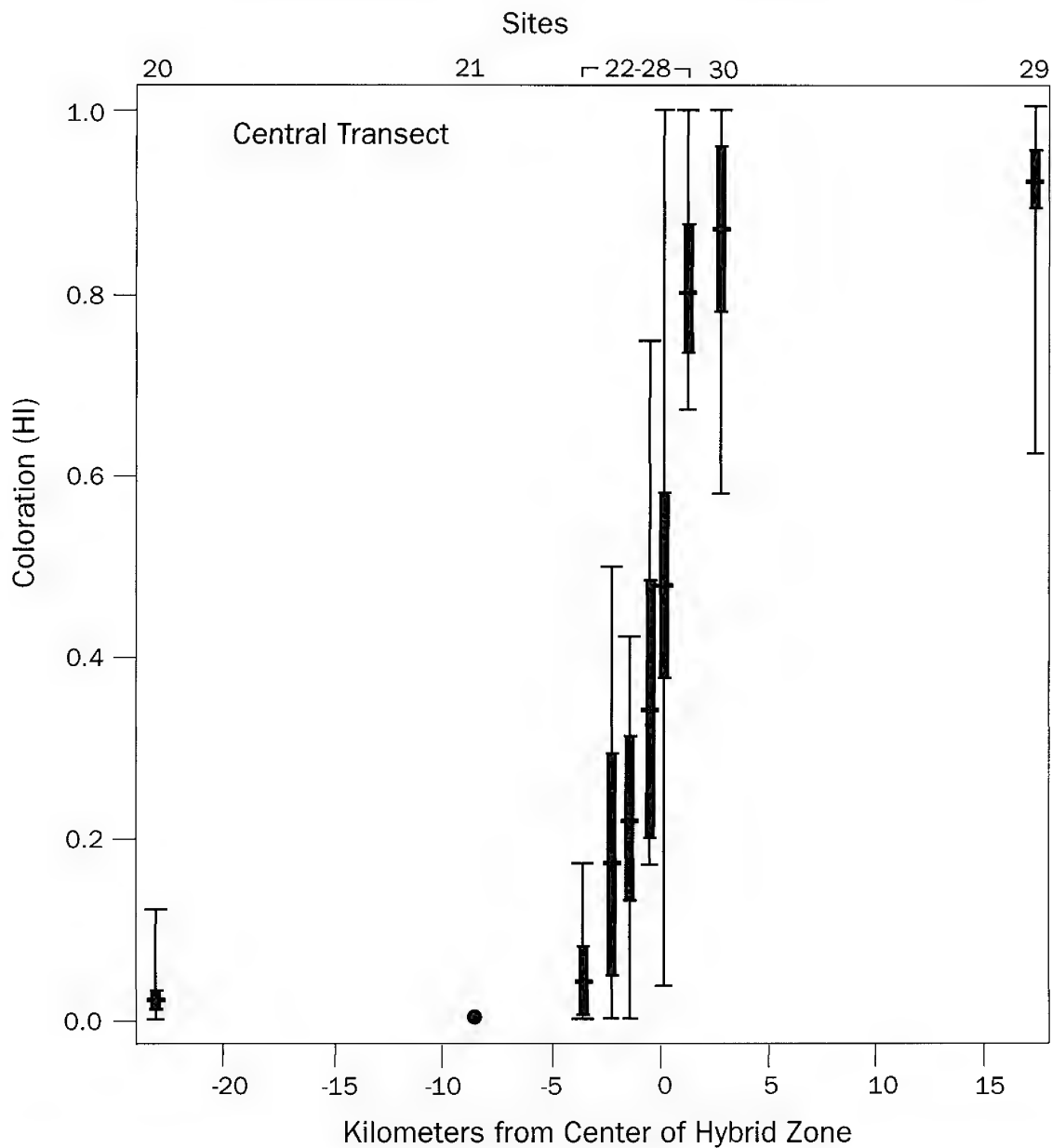
Fig. 37. Frequencies of the *marmoratus* body coloration characters at sites along the central transect (fig. 5). Site 26 was the center of the central hybrid zone in the genetic characters (fig. 31). Site 27 was not included because of crowding. Interpretation as in figure 36, with black spots representing samples in which all individuals were identical.
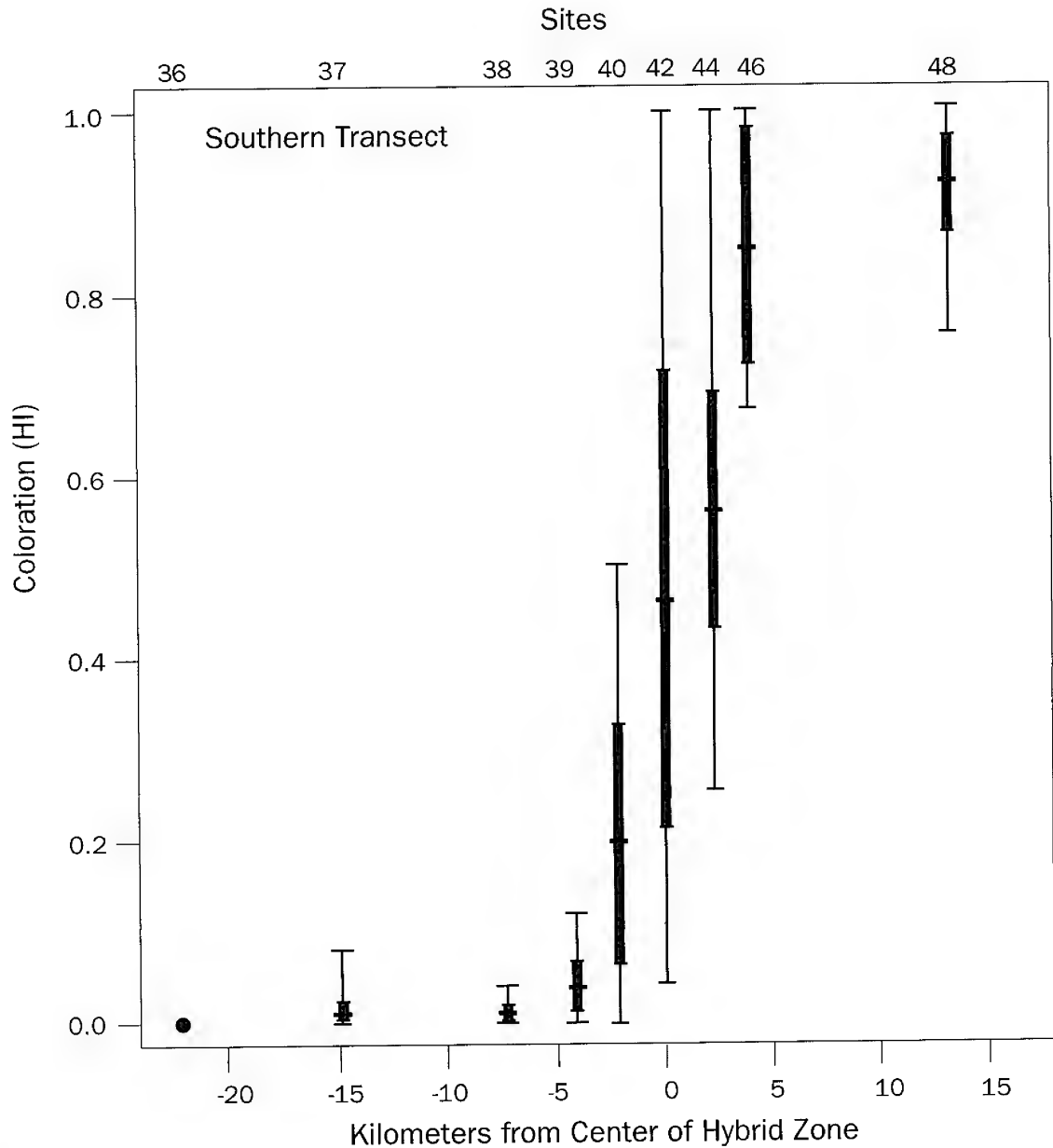
Fig. 38. Frequencies of the *marmoratus* body coloration characters at sites along the southern transect (fig. 5). Site 42 was the center of the southern hybrid zone in genetic characters (fig. 32). Interpretation as in figure 37.

Fig. 39. Mean frequency of the *marmoratus* nuclear alleles averaged over all four diagnostic loci of proteins, of the *marmoratus* 12S ribosomal mtDNA haplotypes, and of the *marmoratus* coloration hybrid indices at sites along the northern transect (fig. 4). Site 3 represents the center of the northern hybrid zone. Compare with figure 30.
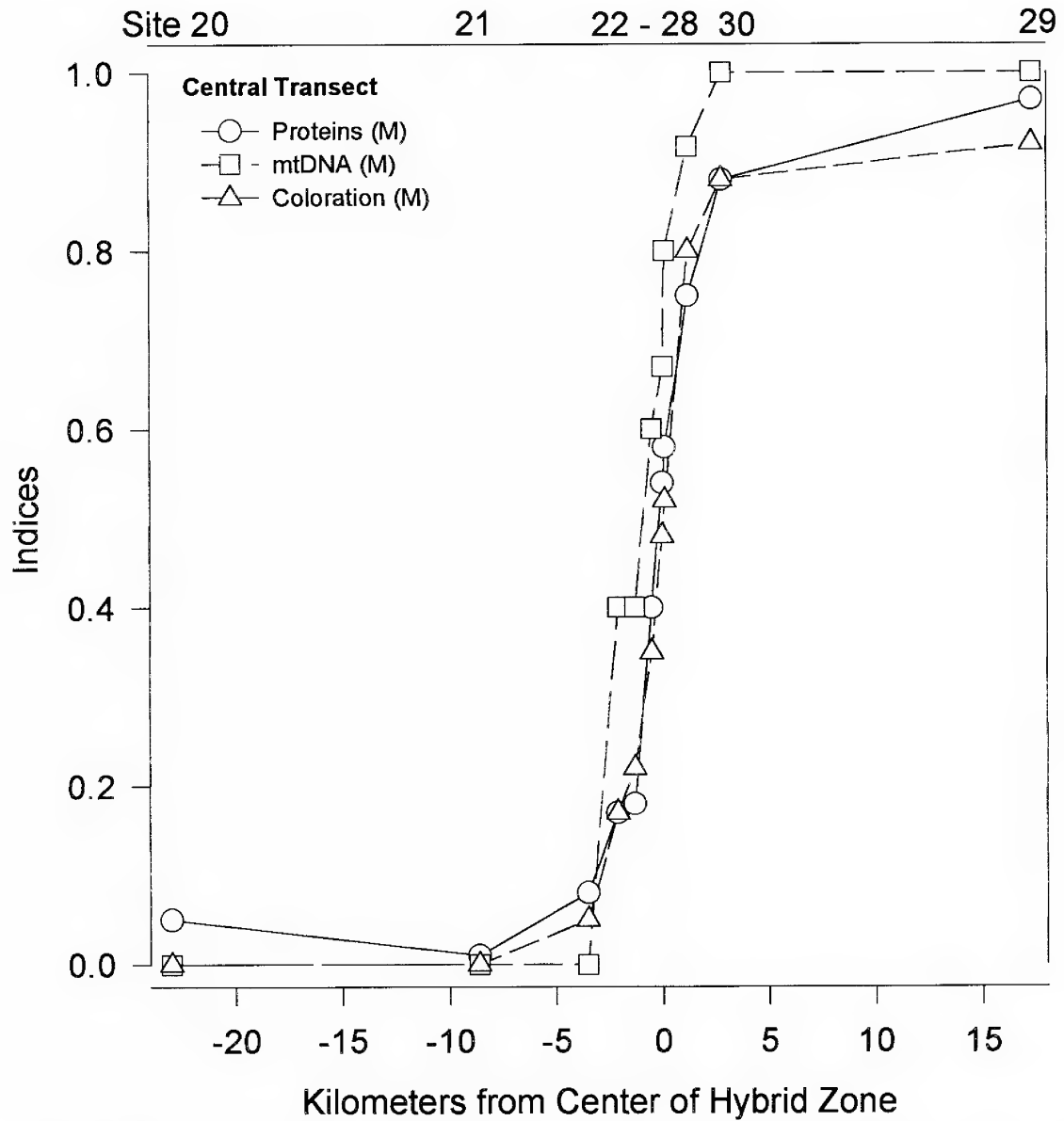
Fig. 40. Mean frequency of the *marmoratus* nuclear alleles averaged over all four diagnostic loci of proteins, of the *marmoratus* 12S ribosomal mtDNA haplotypes, and of the *marmoratus* coloration hybrid indices at sites along the central transect (fig. 5). Site 26 represents the center of the central hybrid zone. Compare with figure 31.

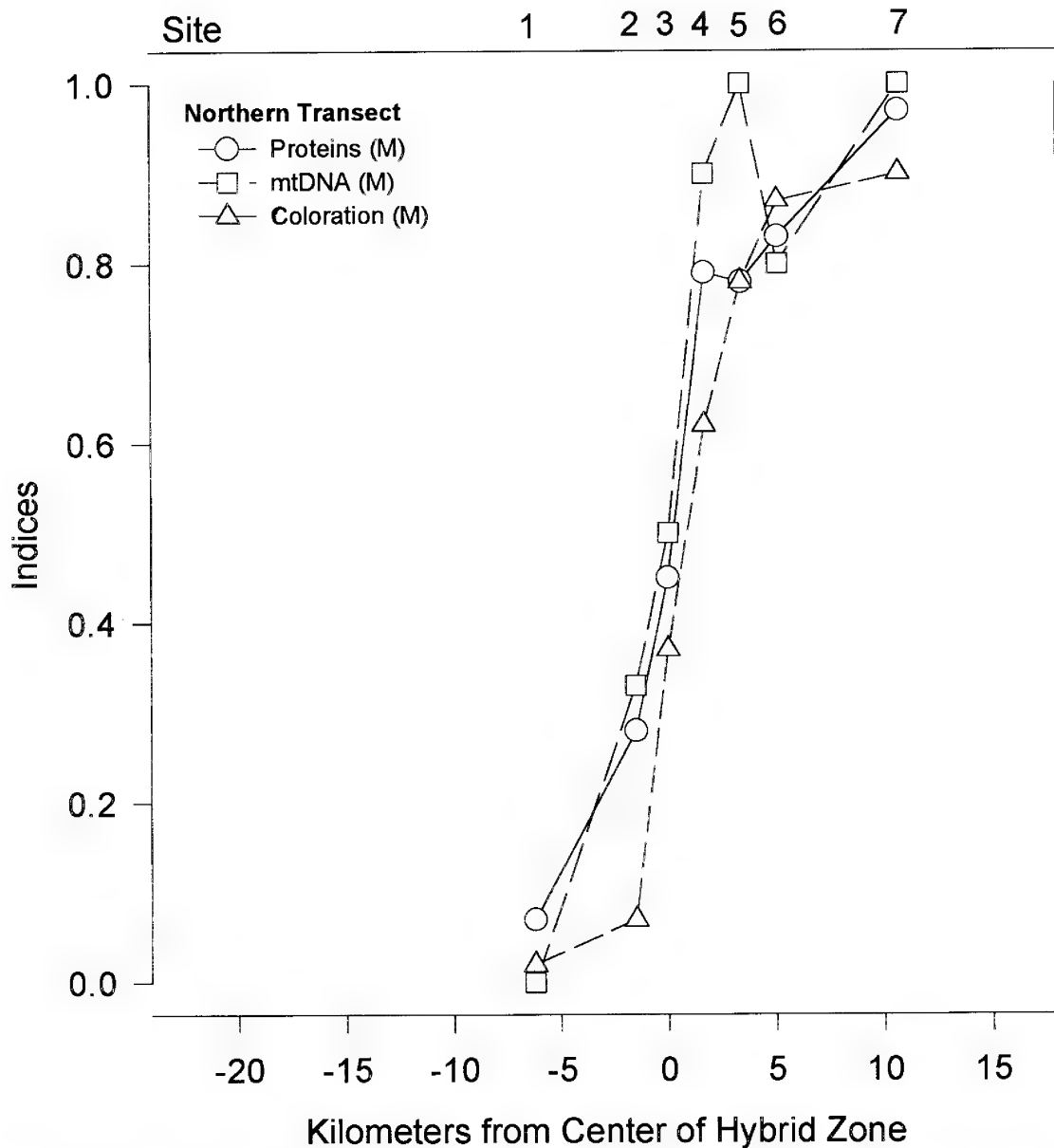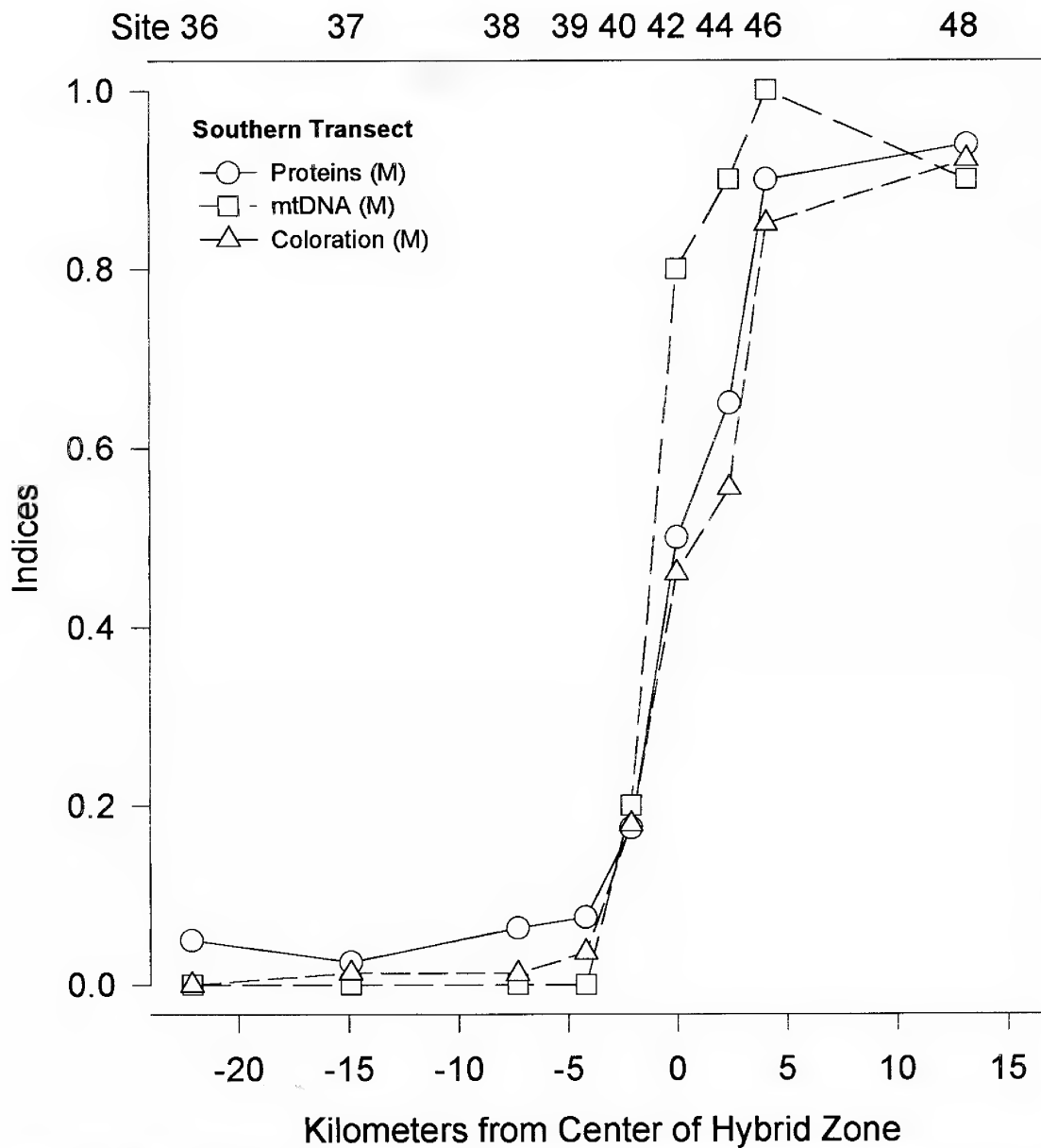Fig. 41. Mean frequency of the *marmoratus* nuclear alleles averaged over all four diagnostic loci of proteins, of the *marmoratus* 12S ribosomal mtDNA haplotypes, and of the *marmoratus* coloration hybrid indices at sites along the southern transect (fig. 5). Site 42 represents the center of the southern hybrid zone. Compare with figure 32.

allele frequencies) we included all three transects on each plot, which emphasizes their similarities.

The PCAs confirm the very high correlation of the variation among the coloration and genetic characters (figs. 42 and 43; table 25). For all six PCAs (separate ones for both coloration and alleles for each of the three transects), the first principal component alone accounted for 93–97% of the total variation in the dataset (table 25), and none of the other PCs explained more than 4%. Thus, more than 90% of the variation in the six coloration characters and in the seven gene loci analyzed is explained by one linear combination of the data. The correlation matrix indicated that all of these characters were correlated with each other on each transect. The loadings on PC1 are presented in table 25. For both the morphological and genetic data (figs. 42 and 43), we interpret the PC1 scores to reflect the distance between the sample locality and pure *C. t. marmoratus*.

Based on coloration characters and somewhat smaller samples, Dessauer and Cole (1991) found no samples within the central transect in which individuals of both pure taxa appeared. Now, based on larger samples and a better understanding of local variation within these taxa, we modify this to recognize that the site of the midpoint of gene exchange in each of the three transects has a few individuals with coloration of nonhybrids as well as numerous obvious hybrids with a large spectrum of variation. Based on the data in table 24, we accept individuals with a hybrid index of 0–0.1 as pure *C. t. punctilinealis*, those with an index of 0.8–1.0 as pure *C. t. marmoratus*, and those with an index of 0.4–0.6 as being perhaps F_1 hybrids. Comparing tables 24 and 26 (sample sizes are in table 24), we find the following: (1) for the northern transect, only site 3 had both apparently pure taxa and hybrids, with pure *punctilinealis* occurring only from site 3 toward the north, and pure *marmoratus* occurring only from site 3 toward the south; (2) for the central transect, only site 26 had both apparently pure taxa and hybrids, with pure *punctilinealis* occurring only from site 26 toward the west, and pure *marmoratus* occurring only from site 26 toward the east; and (3) for the southern transect, only site 42 had both apparently pure taxa and hybrids, with pure *punctilinealis* occurring only from site 42 toward the west, and pure *marmoratus* occurring only from site 42 toward the east.

Finally, it is illuminating to compare the separate genetic characters and coloration characters as they occurred together on individual lizards from the midpoints of the three hybrid zones. On the basis of the four diagnostic protein loci, there appeared to be a total of four pure (nonhybrid) lizards from sites 3 and 26 only (table 13), and on the basis of coloration, there appeared to be a total of 14 nonhybrids from sites 3, 26, and 42 (table 26). The coloration and genetic characters of these so-called nonhybrid individuals are compared together on a specimen-by-specimen basis in table 27.

For sites 3 ($N = 4$), 26 ($N = 9$), and 42 ($N = 4$), a total of 17 specimens appeared to be nonhybrids based on either coloration (HI) or protein data (table 27). Of these 17 lizards, only two (FT 6727 and 1619) appeared to be nonhybrids when their morphological and genetic data were considered together, whereas 15 lizards were revealed to be hybrids. As mentioned above, the protein data were more sensitive and reliable than the morphological data, as 12 specimens that appeared morphologically to be nonhybrids had a combination of protein alleles from both subspecies. Five specimens had a nonhybrid appearance in morphology but the mtDNA haplotype of the opposite subspecies. In addition, one lizard (FT 1621) had all four diagnostic protein alleles of one form, the mtDNA haplotype of the opposite form, and a hybrid coloration phenotype.

In summary, examination of all characters on an individual specimen basis (table 27) is consistent with our other data indicating that these hybrid zones involve panmixia, nonassortative mating, and an absence of selection against hybrids.

BODY SIZE AND SCALATION: Body measurement and epidermal scale count data are presented for nine characters in table 28 (nine characters when counting each paired character as one). Additional characters examined showed no significant differences between *C. t. punctilinealis* and *C. t. marmoratus* or their hybrids (with no significant bilateral asymmetry also) as follows: two fron-
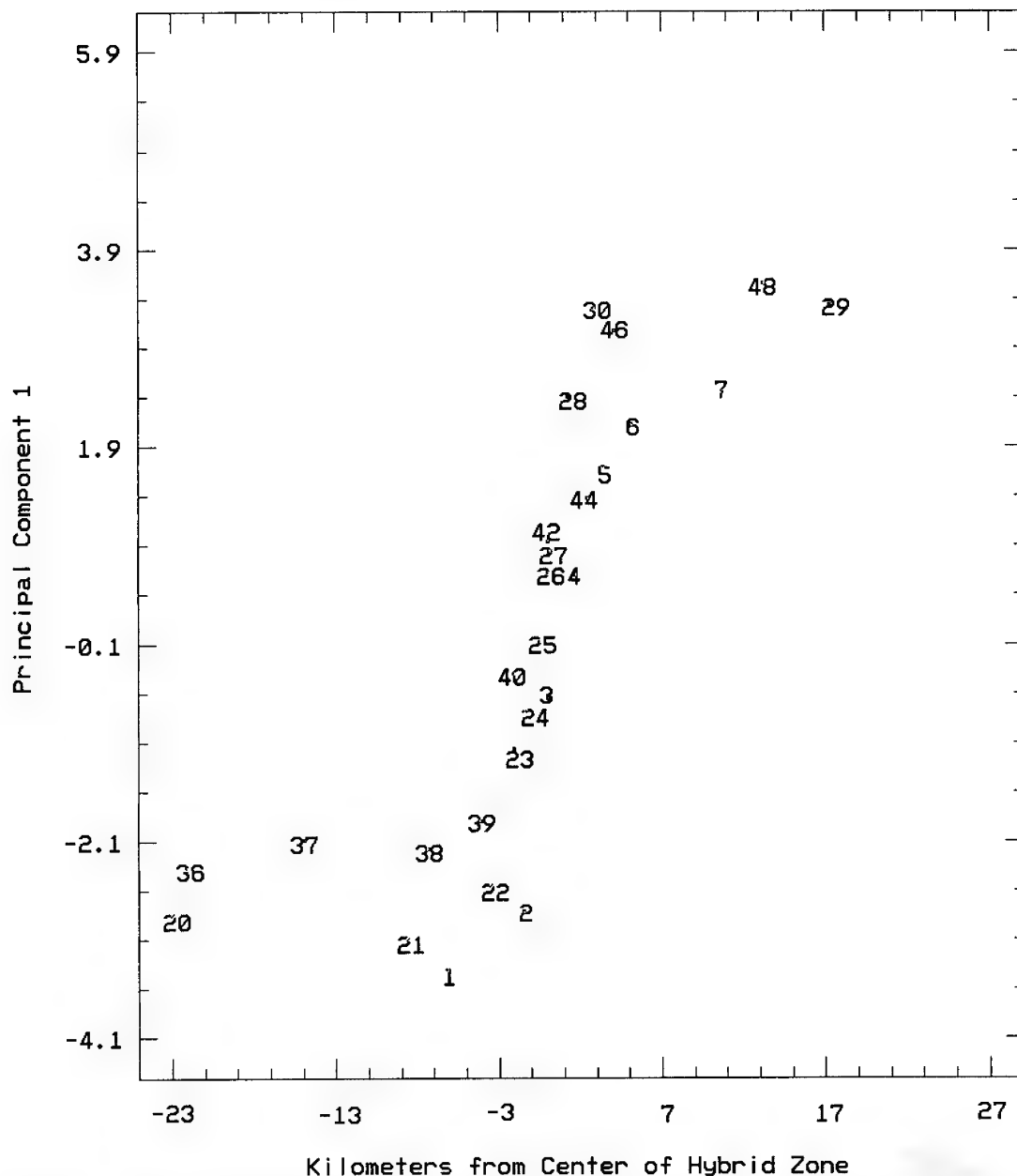
Fig. 42. Scores of sample means for the first principal component extracted from the variance–covariance matrix of arcsine square-root-transformed frequencies of *marmoratus* coloration characters plotted against sample locality on all three transects of the hybrid zones: northern (sites 1–7), central (sites 20–30 + 29), and southern (sites 36–40, 42, 44, 46, and 48). Numbers plotted are the collecting site numbers. Data are summarized in table 24.
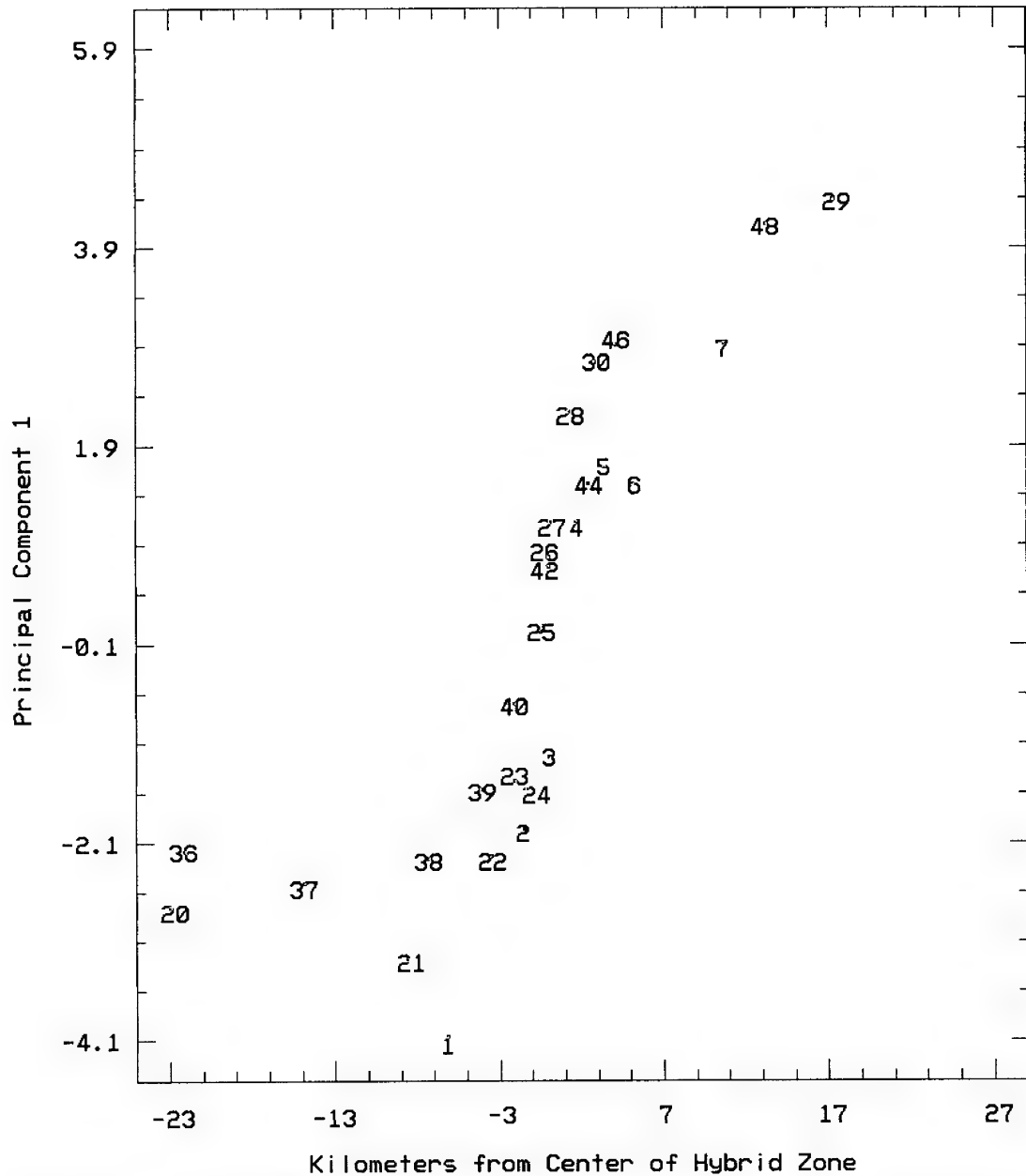
Fig. 43. Scores of sample means for the first principal component extracted from the variance–covariance matrix of arcsine square-root-transformed frequencies of *marmoratus* alleles at the seven highly polymorphic loci (IDDH, sMDHP, EST2, PEPB, PEPD, GPI, and TF) plotted against sample locality on all three transects of the hybrid zones, as in figure 42. Data are from tables 6, 8, and 10.

TABLE 25

Character Loadings and Variation Explained for the First Axis of Principal Components Analyses of Coloration and Allelic Indices of *Cnemidophorus tigris* from the Northern (NPC1), Central (CPC1), and Southern (SPC1) Transects

Character	NPC1	CPC1	SPC1
		Coloration	
Dorsal stripes	0.382	0.327	0.346
Dorsal spots	0.420	0.361	0.336
Lateral bars	0.321	0.314	0.308
Throat	0.353	0.418	0.448
Chest	0.376	0.468	0.494
Abdomen	0.556	0.520	0.477
Variation explained	97%	95%	94%
		Alleles	
IDDHa	0.272	0.190	0.193
sMDHPa	0.410	0.420	0.459
EST2a	0.415	0.415	0.311
PEPBc, d	0.470	0.458	0.463
PEPDc	0.257	0.330	0.270
GPIa, b	0.302	0.257	0.340
TFa	0.455	0.480	0.502
Variation explained	93%	95%	93%

toparietals, three parietals, four supraoculars per side, eight rows of ventrals across midbody (discussed further below), mesoptychial scales not abruptly enlarged, postantebrachials granular, seven supralabials per side (to posterior corner of eye), zero suprasuprala-

bials per side, six enlarged genials per side, and preanal scales usually type I (also discussed further below).

Data for the nine rather variable characters discussed in more detail below are presented for 252 specimens in table 28. These were analyzed to address questions concerning sexual dimorphism, bilateral symmetry, and comparisons of the pure taxa with the hybrids.

Sexual dimorphism: For the largest samples (sites 14 [nonhybrids], 20 [nonhybrids], 26 [hybrids], and 29 [nonhybrids]), of which site 26 is the midpoint of gene exchange for the central transect (i.e., hybrids), we used two-sided t-tests to analyze males versus females for sexual dimorphism. This required 60 separate tests for the four sites and the full suite of characters in table 28. Of these tests, 56 indicated no sexual dimorphism. The few suggestions of sexual dimorphism are discussed immediately below.

The largest sample (site 14) showed no significant difference ($P > 0.05$) in body size of males and females, whereas the smaller samples (sites 20, 26, and 29) did ($P < 0.05$). The large sample was collected over the longest period of time and consequently may have less sampling bias, so we accept the suggestion that body size is not significantly sexually dimorphic in *C. tigris*, although it is in some other species of *Cnemidophorus* (see

TABLE 26

Number of Lizards of "Pure" Parental Forms and F₁ Hybrid Form at Sites Across the Three Transects as Judged From Six Characters of Coloration

Hybrid index[a]	Site										
Northern Transect Site	1	2	**3**	4	5	6	7				
Pure PUN (0.0–0.1)	10	6	**2**	0	0	0	0				
F₁ hybrid (0.4–0.6)	0	0	**4**	4	2	0	1				
Pure MAR (0.8–1.0)	0	0	**1**	2	4	6	9				
Central Transect Site	20	21	22	23	24	25	**26**	27	28	30	29
Pure PUN (0.0–0.1)	31	10	9	6	3	0	**2**	0	0	0	0
F₁ hybrid (0.4–0.6)	0	0	0	2	2	2	**6**	6	0	1	0
Pure MAR (0.8–1.0)	0	0	0	0	0	0	**5**	0	5	8	28
Southern Transect Site	36	37	38	39	40	**42**	44	46	48		
Pure PUN (0.0–0.1)	10	10	10	9	4	**2**	0	0	0		
F₁ hybrid (0.4–0.6)	0	0	0	0	2	**1**	6	0	0		
Pure MAR (0.8–1.0)	0	0	0	0	0	**2**	1	7	8		

[a] For sites where the total number of specimens does not equal the sample size (table 24), the specimens excluded here had hybrid indices of 0.11–0.39 or 0.61–0.79. Sites 3, 26, and 42 are the 50:50 points of gene exchange (midpoints) of their respective transects.

TABLE 27

Comparison of Coloration, Protein Alleles, and Mitochondrial DNA Haplotypes of Apparently Nonhybrid Individuals from the Midpoints of the Three Hybrid Zones[a]

FT no.[b]	HI	Proteins	mtDNA
		Site 3	
2030	PUN	HYB	PUN
2061	PUN	HYB	PUN
6727	MAR	MAR	MAR
6724	HYB	PUN	PUN
		Site 26	
1623	PUN	HYB	MAR
1624	PUN	HYB	PUN
1631	MAR	HYB	PUN
6281	MAR	HYB	MAR
6730	MAR	HYB	MAR
6732	MAR	HYB	MAR
1619	MAR	MAR	MAR
1621	HYB	PUN	MAR
6279	HYB	MAR	MAR
		Site 42	
0668	PUN	HYB	MAR
0669	PUN	HYB	MAR
0673	MAR	HYB	PUN
0675	MAR	HYB	MAR

[a] The collecting sites and hybrid zones are specified and mapped in table 2 and figures 3–5 and 49.

[b] Frozen tissue collection number and data for each specimen are presented in appendix 3. HI = coloration hybrid index; proteins = nuclear alleles present at the four diagnostic loci (sMDHP, EST2, PEPB, and TF); and mtDNA = haplotype of mitochondrial DNA. HYB indicates a condition mixed between pure traits of *C. t. marmoratus* (MAR) and pure *C. t. punctilinealis* (PUN).

Markezich et al. [1997] regarding species in the South American *C. lemniscatus* complex). There is no taxonomic bias indicated here, as the conflicting results with small versus large samples both included samples of pure *C. t. marmoratus* (sites 14 and 29). The range of variation observed in the specimens from each of these four sites is similar (table 28), but we do note that the mean body size of the males appears larger than that of the females in each sample (although not being statistically significant for site 14).

The number of infralabials on the right side appeared sexually dimorphic ($P < 0.05$) for site 26 only. We do not attach special significance to this because none of the other sites had dimorphic samples for right infralabials ($P > 0.05$), and the number of infralabials on the left side was not sexually dimorphic in any sample. Note also (table 28) that in the sample of each sex for site 26, the mean would be rounded to eight for the nearest whole scale, and the observed ranges were nearly identical also.

The coloration hybrid index was also tested for sexual dimorphism in the largest sample of hybrids (site 26, central transect), and no difference was indicated ($P > 0.05$).

We concluded that there is no significant sexual dimorphism in these morphological characters and therefore conducted further tests involving size, scalation, and coloration with the sexes pooled.

Comparisons (size and scalation) of the pure taxa and hybrids: Inspection of the data in table 28 (lower section with sexes pooled) suggests the following for *C. t. punctilinealis* (P), *C. t. marmoratus* (M), and their hybrids (H), using the samples from the terminal pure populations and the center (midpoint of gene exchange) of each of the three transects: (1) the samples are rather similar to each other, showing extensive overlap in the range of variation of each character of size and scalation; (2) the mean number of circumorbital scales is higher in *marmoratus* than in *punctilinealis* and is intermediate in hybrids; (3) the mean number of femoral pores is higher in *marmoratus* than in *punctilinealis* and is intermediate in hybrids; and (4) no other characters are significantly different between *punctilinealis* and *marmoratus*.

Principal components analyses (PCAs) were performed with three samples (sexes pooled) from each transect, based on the data summarized in table 28. Because the pure taxa and their hybrids are so similar in size and scalation, we analyzed only the hybrids from the midpoint of gene exchange in each transect and the extreme terminal pure samples of each transect (*punctilinealis* sites 1, 20, and 36; *marmoratus* sites 7, 29, and 48). Inspection of the data suggested that inclusion of all samples for each transect would have produced plots with a smear of scores blending from one form into the other. This is in striking contrast to the PCA plots for morphological characters of unisexual species of hybrid or-

TABLE 28
External Morphological Data for *Cnemidophorus tigris*

Site[a]	Ident.[b]	Sex	Body length[c]	L circum-orbitals[d]	R circum-orbitals[d]	L infra-labials	R. infra-labials	L gulars
14	M	♀	84.7±0.88	11.0±0.33	10.9±0.29	7.8±0.12	8.0±0.15	10.9±0.18
			(70–92)34	(6–15)34	(8–15)34	(6–9)34	(6–10)34	(9–13)28
14	M	♂	86.9±0.97	10.7±0.23	10.8±0.23	7.8±0.09	8.0±0.11	11.0±0.17
			(62–96)54	(6–15)53	(7–14)52	(6–9)54	(7–10)54	(9–14)43
20	P	♀	76.0±1.94	8.2±0.42	8.3±0.33	7.2±0.20	7.6±0.34	11.6±0.50
			(66–89)10	(7–11)10	(7–10)10	(6–8)10	(6–9)10	(10–13)8
20	P	♂	82.0±1.34	7.5±0.24	7.6±0.25	7.6±0.12	7.7±0.15	11.4±0.23
			(70–91)22	(5–11)22	(6–11)22	(7–9)22	(6–9)22	(9–13)21
26	H	♀	76.1±1.90	9.2±0.61	9.3±0.62	7.8±0.22	8.2±0.18	11.4±0.25
			(65–86)12	(5–13)12	(5–14)12	(7–9)12	(7–9)12	(10–13)11
26	H	♂	84.4±1.62	8.7±0.47	8.4±0.45	7.4±0.17	7.5±0.18	11.0±0.32
			(68–95)19	(6–13)19	(6–12)19	(6–9)19	(6–9)19	(8–14)18
29	M	♀	75.7±1.88	10.6±0.48	10.3±0.42	7.8±0.15	8.1±0.20	10.5±0.31
			(64–86)14	(8–14)14	(8–13)14	(7–9)13	(7–9)14	(9–12)11
29	M	♂	82.8±1.16	10.5±0.43	10.8±0.44	7.6±0.19	8.0±0.15	10.6±0.23
			(71–92)17	(6–13)17	(7–14)17	(7–9)17	(7–9)17	(9–12)14
14	M	♂ + ♀	86.1±0.69	10.8±0.19	10.8±0.17	7.8±0.07	8.0±0.09	10.9±0.12
			(62–96)88	(6–15)87	(7–15)86	(6–9)88	(6–10)88	(9–14)71
1	P	♂ + ♀	81.6±2.36	7.8±0.55	7.9±0.62	7.4±0.29	7.4±0.16	12.5 ± 0.31
			(70–92)10	(5–11)9	(5–12)10	(6–8)9	(7–8)10	(11–14)10
3	H	♂ + ♀	83.3±1.75	7.8±0.35	7.7±0.37	7.9±0.16	8.1±0.16	11.8±0.21
			(67–96)20	(5–11)20	(5–11)20	(7–10)20	(7–9)20	(10–14)19
7	M	♂ + ♀	84.3±2.08	9.7±0.58	9.8±0.53	8.2±0.28	8.2±0.25	10.8±0.49
			(68–92)10	(6–12)10	(7–12)10	(7–9)9	(7–9)10	(8–12)9
20	P	♂ + ♀	80.1±1.19	7.8±0.22	7.8±0.21	7.5±0.11	7.7±0.14	11.5±0.21
			(66–91)32	(5–11)32	(6–11)32	(6–9)32	(6–9)32	(9–13)29
26	H	♂ + ♀	81.2±1.42	8.9±0.37	8.7±0.37	7.5±0.14	7.8±0.14	11.2±0.22
			(65–95)31	(5–13)31	(5–14)31	(6–9)31	(6–9)31	(8–14)29
29	M	♂ + ♀	79.6±1.23	10.5±0.31	10.6±0.31	7.7±0.13	8.0±0.12	10.6±0.18
			(64–92)31	(6–14)31	(7–14)31	(7–9)30	(7–9)31	(9–12)25
36	P	♂ + ♀	86.3±1.69	6.9±0.40	7.1±0.35	7.3±0.21	7.1±0.10	11.1±0.31
			(76–92)10	(5–8)8	(6–9)9	(6–8)10	(7–8)10	(10–13)9
42	H	♂ + ♀	86.4±3.19	9.5±0.67	8.9±0.62	7.5±0.17	7.5±0.27	12.0±0.62
			(67–97)10	(7–14)10	(7–14)10	(7–8)10	(6–9)10	(10–15)9
48	M	♂ + ♀	77.9±1.80	10.7±0.52	11.2±0.62	7.8±0.20	8.0±0.26	11.0±0.39
			(70–86)10	(8–14)10	(9–15)9	(7–9)10	(7–9)10	(9–13)10

[a] Sites are listed in table 2 and mapped in figures 3–5. Sites 3, 26, and 42 are the 50:50 points of gene exchange (midpoints) of their respective transects. Appendix 1 presents complete locality data, descriptions of habitats, and list of specimens examined.

[b] M = *C. t. marmoratus*; P = *C. t. punctilinealis*; and H = hybrid.

igin compared with their bisexual ancestors (Cole et al., 1988, 1995). In those cases, the bisexual ancestors are more distantly related and morphologically distinct (no overlap in PC1 scores), and the unisexual clone is distinctly intermediate, with apparently less variation than its ancestors.

Results of the three PCAs (one for each transect) are shown in figures 44–46 and table 29 (character loadings on the first five axes and variation explained by each). For the northern and central transects, particularly the latter, which has the largest samples, there was overlap in scores on PC1 and PC2 for both pure forms, and the hybrid sample showed a broad range of variation that en-

TABLE 28—(Extended)

R gulars	Scales around midbody	L femoral pores	R femoral pores	Inter-femorals	L toe lamellae	R toe lamellae	L finger lamellae	R finger lamellae
11.0±0.16	91.8±0.89	21.6±0.20	21.4±0.25	4.2±0.12	33.0±0.26	32.8±0.27	16.6±0.14	16.7±0.14
(9–13)29	(83–102)34	(19–24)34	(18–26)34	(2–6)34	(30–36)34	(29–36)32	(15–18)32	(15–18)30
10.6±0.14	91.8±0.91	21.2±0.19	20.9±0.19	4.0±0.08	32.4±0.26	32.2±0.26	16.9±0.14	16.6±0.15
(9–12)42	(80–110)52	(18–24)54	(17–23)53	(3–6)54	(28–36)53	(28–35)54	(15–19)51	(12–19)53
12.1±0.67	89.3±2.10	18.4±0.30	18.1±0.35	4.5±0.22	30.7±0.45	31.0±0.62	16.7±0.29	15.8±0.42
(9–14)7	(82–99)9	(17–20)10	(16–19)10	(4–6)10	(28–32)10	(28–34)9	(15–18)9	(14–18)10
11.3±0.25	88.2±1.09	17.9±0.29	18.0±0.28	4.4±0.17	31.0±0.27	30.7±0.39	16.3±0.21	15.8±0.19
(9–13)22	(79–97)21	(16–21)22	(16–21)22	(3–6)22	(28–33)22	(28–34)21	(14–18)22	(15–17)21
11.2±0.41	90.8±2.30	19.6±0.50	19.9±0.48	4.8±0.22	30.7±0.54	30.6±0.53	16.8±0.27	16.8±0.25
(9–14)12	(80–105)12	(16–22)12	(17–22)12	(4–6)12	(29–35)11	(27–33)12	(15–18)12	(16–18)12
10.7±0.21	90.0±1.29	20.6±0.40	20.3±0.32	4.5±0.16	31.5±0.54	31.2±0.64	16.8±0.23	16.8±0.31
(9–12)18	(81–100)18	(18–25)19	(18–23)19	(4–6)19	(27–37)18	(26–37)18	(15–19)19	(15–19)19
10.7±0.33	88.0±1.60	21.0±0.44	20.6±0.44	4.1±0.13	31.9±0.56	31.5±0.55	17.1±0.33	16.4±0.31
(9–12)11	(77–98)14	(18–23)14	(18–24)14	(3–5)14	(29–36)13	(28–35)14	(15–19)14	(15–19)14
10.7±0.28	89.4±1.48	21.1±0.22	21.4±0.30	4.4±0.21	32.2±0.37	31.6±0.49	16.9±0.14	16.6±0.18
(9–13)15	(78–102)17	(19–22)17	(19–23)16	(3–6)17	(30–35)16	(27–34)14	(16–18)17	(15–18)16
10.8±0.11	91.8±0.65	21.3±0.14	21.1±0.15	4.1±0.07	32.6±0.19	32.4±0.20	16.8±0.10	16.6±0.11
(9–13)71	(80–110)86	(18–24)88	(17–26)87	(2–6)88	(28–36)87	(28–36)86	(15–19)83	(12–19)83
12.2±0.42	93.1±1.48	17.6±0.22	18.3±0.37	4.9±0.23	32.1±0.52	31.5±0.31	16.7±0.26	16.8±0.22
(11–15)10	(85–100)10	(17–19)10	(17–20)10	(4–6)10	(30–35)10	(30–33)10	(15–18)10	(16–18)9
11.2±0.24	91.0±0.84	19.5±0.37	19.4±0.38	4.4±0.13	31.2±0.44	30.8±0.45	16.4±0.18	16.1±0.19
(9–13)18	(84–97)20	(16–23)20	(16–22)20	(3–5)20	(28–34)20	(28–34)19	(15–18)20	(15–18)20
10.9±0.39	87.8±1.55	21.1±0.35	21.1±0.57	3.8±0.13	31.4±0.29	31.4±0.20	16.7±0.26	16.7±0.33
(9–12)9	(81–96)10	(19–23)10	(18–24)10	(3–4)10	(30–33)9	(31–32)7	(15–18)10	(15–19)10
11.5±0.25	88.6±0.97	18.1±0.22	18.1±0.22	4.5±0.13	30.9±0.23	30.8±0.33	16.4±0.17	15.8±0.18
(9–14)29	(79–99)30	(16–21)32	(16–21)32	(3–6)32	(28–33)32	(28–34)30	(14–18)31	(14–18)31
10.9±0.21	90.4±1.18	20.2±0.32	20.2±0.27	4.6±0.13	31.2±0.40	30.9±0.43	16.8±0.17	16.8±0.21
(9–14)30	(80–105)30	(16–25)31	(17–23)31	(4–6)31	(27–37)29	(26–37)30	(15–19)31	(15–19)31
10.7±0.21	88.8±1.07	21.1±0.23	21.1±0.27	4.2±0.13	32.1±0.32	31.5±0.36	17.0±0.17	16.5±0.17
(9–13)26	(77–102)31	(18–23)31	(18–24)30	(3–6)31	(29–36)29	(27–35)28	(15–19)31	(15–19)30
10.4±0.41	89.0±2.36	18.2±0.39	18.4±0.27	4.2±0.13	31.4±0.50	31.3±0.50	16.0±0.33	16.0±0.33
(9–13)9	(77–102)10	(16–20)10	(17–20)10	(4–5)10	(28–33)10	(29–33)10	(14–17)10	(14–17)10
11.8±0.49	91.2±1.40	19.4±0.50	19.6±0.41	4.6±0.22	31.7±0.52	31.4±0.72	17.1±0.38	16.4±0.40
(10–14)8	(84–98)10	(17–21)9	(17–21)9	(4–6)10	(29–35)10	(28–35)10	(15–19)10	(15–18)10
11.3±0.40	89.3±1.57	21.8±0.33	21.6±0.37	3.9±0.26	32.6±0.41	32.2±0.46	16.5±0.17	16.5±0.27
(9–14)10	(83–97)10	(20–23)10	(20–24)10	(3–5)9	(31–35)9	(30–34)9	(16–17)10	(15–17)10

[c] Mean ± 1 standard error of the mean (range) and sample size. Body length (snout–vent) is in mm; all other data are discrete scale counts.

[d] L = left side of body; R = right side; tested for left/right asymmetry in largest samples (sites 14, 20, and 29, as well as hybrid sites 3, 26, and 42).

compassed nearly all of the extreme scores, as one would predict for a panmictic hybrid swarm. For the southern transect (fig. 46), the pure forms showed no overlap in scores on PC1, whereas the hybrids tended to be intermediate but with a broad overlap with *C. t. punctilinealis*. Given the sensitivity of PCAs and the variation in our sample sizes,

we suspect that the northern and southern transects (figs. 44 and 46) would have appeared more similar to the central transect (fig. 45) if we had had equally large samples for them.

Similar percentages of variation were explained by PC1–PC5 for each of the three transects (table 29). PC1 never explained
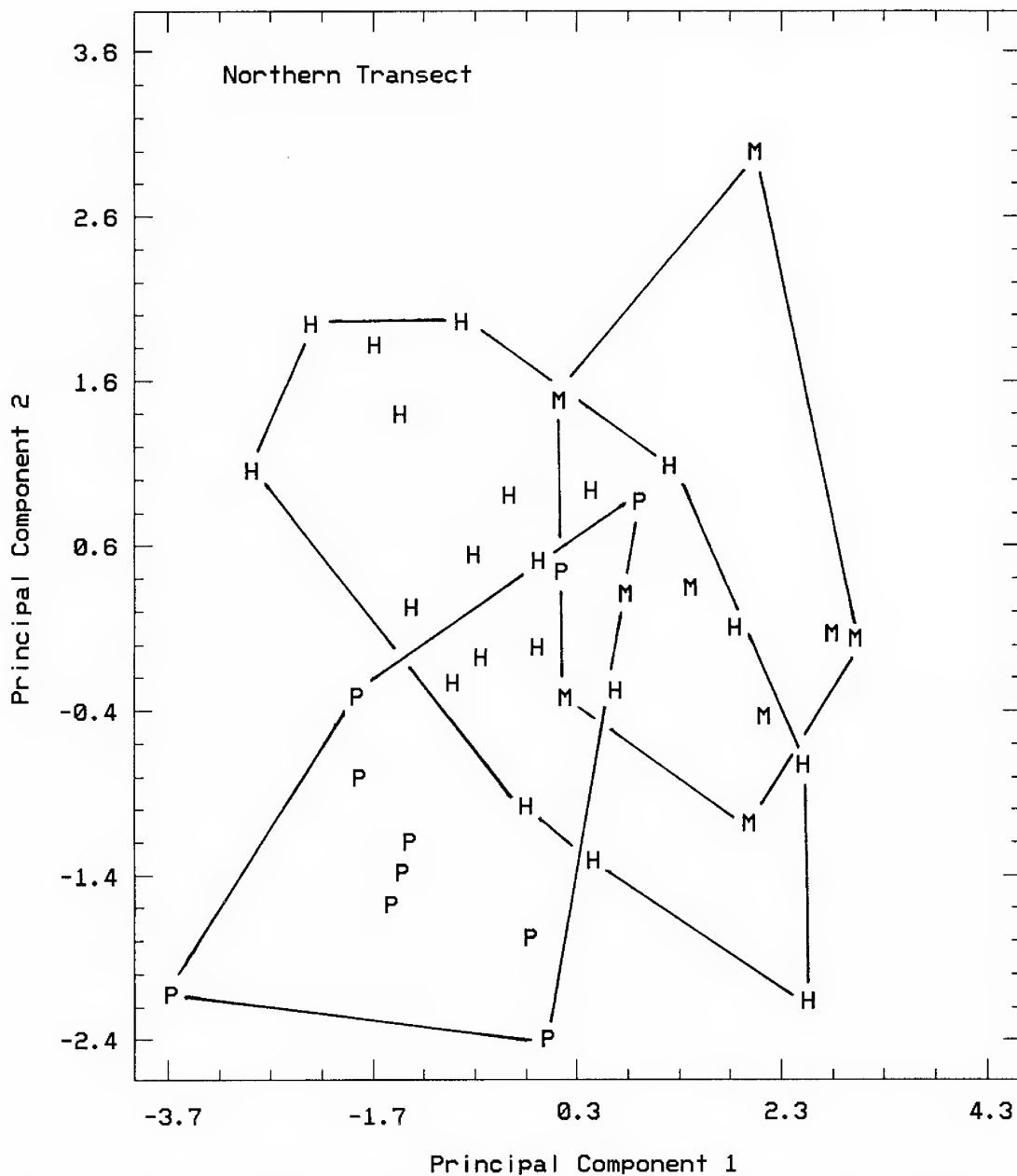
Fig. 44.   Polygons and letters representing the scores of 39 specimens of *C. tigris* on the first two principal components extracted from the correlation matrix of nine morphological characters observed in the northern transect (table 28). P represents 10 *punctilinealis* from site 1; M, nine *marmoratus* from site 7; and H, 20 hybrids from site 3, the center of the northern hybrid zone (fig. 4).
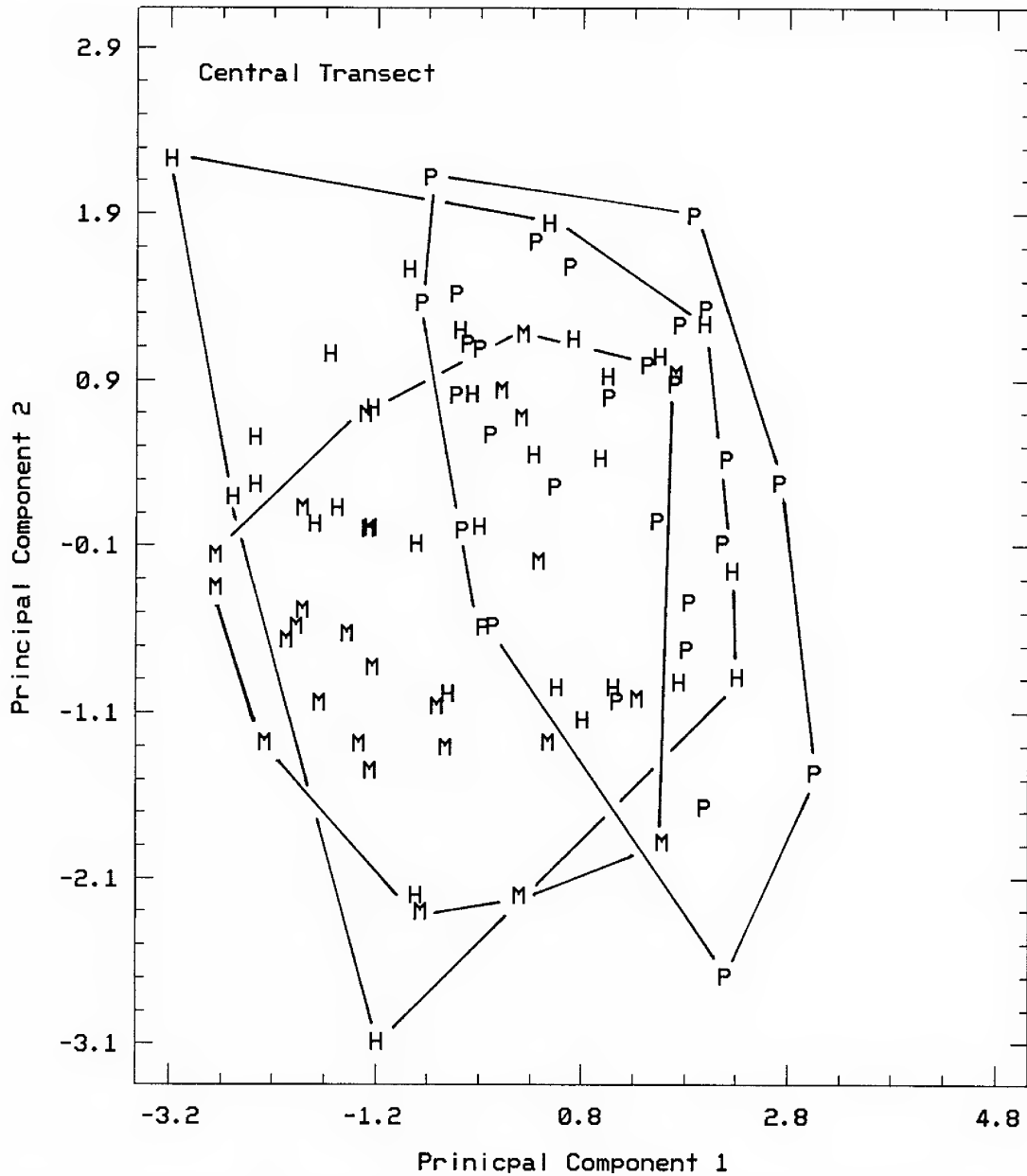
Fig. 45. Polygons and letters representing the scores of 85 specimens of *C. tigris* on the first two principal components extracted from the correlation matrix of nine morphological characters observed in the central transect (table 28). P represents 29 *punctilinealis* from site 20; M, 26 *marmoratus* from site 29; and H, 30 hybrids from site 26, the center of the central hybrid zone (fig. 5).
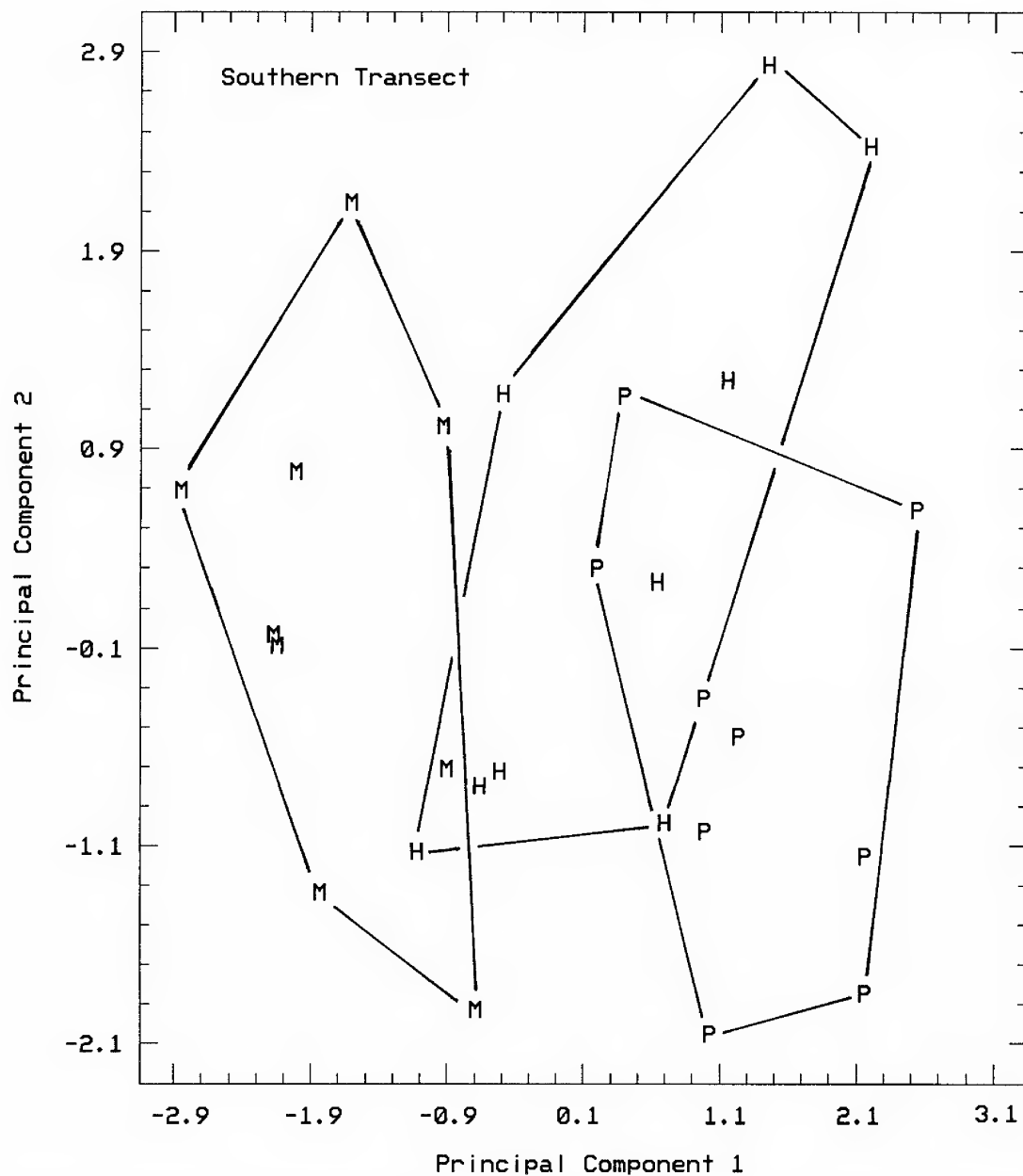
Fig. 46. Polygons and letters representing the scores of 27 specimens of *C. tigris* on the first two principal components extracted from the correlation matrix of nine morphological characters observed in the southern transect (table 28). P represents 9 *punctilinealis* from site 36; M, 9 *marmoratus* from site 48; and H, 9 hybrids from site 42, the center of the southern hybrid zone (fig. 5).

TABLE 29
Character Loadings and Variation Explained for the First Five Axes of the Principal Components Analyses of Size and Scalation Characters of *Cnemidophorus tigris* from Three Hybrid Zones

Character	PC1	PC2	PC3	PC4	PC5
			Northern Transect		
Body length	0.282	−0.054	0.404	−0.692	0.252
Circumorbitals	0.470	0.036	−0.014	−0.076	0.446
Infralabials	0.204	0.158	0.544	0.590	0.220
Gulars	−0.370	−0.202	−0.144	0.220	0.725
Scales around midbody	−0.115	−0.523	0.437	0.137	−0.333
Femoral pores	0.478	−0.063	−0.007	0.276	−0.020
Interfemoral scales	−0.366	−0.310	0.392	−0.145	0.181
Toe lamellae	0.354	−0.486	−0.045	−0.055	−0.096
Finger lamellae	0.142	−0.566	−0.415	0.022	0.099
Variation expl. (79%)	30%	17%	14%	10%	8%
			Central Transect		
Body length	−0.003	0.427	−0.550	−0.292	0.199
Circumorbitals	−0.430	−0.368	−0.153	0.148	−0.247
Infralabials	−0.136	−0.550	−0.122	0.114	0.621
Gulars	−0.030	0.465	0.228	0.697	0.034
Scales around midbody	−0.306	0.286	0.194	−0.522	0.044
Femoral pores	−0.402	−0.066	−0.308	0.050	−0.601
Interfemoral scales	−0.164	−0.118	0.688	−0.255	−0.097
Toe lamellae	−0.520	0.220	0.043	0.223	0.220
Finger lamellae	−0.493	0.124	0.013	−0.069	0.303
Variation expl. (74%)	25%	15%	13%	11%	10%
			Southern transect		
Body length	0.364	0.173	0.477	0.156	−0.489
Circumorbitals	−0.414	0.302	0.073	−0.295	0.351
Infralabials	−0.371	0.155	−0.119	−0.389	−0.457
Gulars	−0.054	0.584	0.115	0.139	−0.228
Scales around midbody	−0.163	0.351	0.584	0.153	0.348
Femoral pores	−0.484	0.142	−0.206	0.112	−0.347
Interfemoral scales	0.254	−0.001	0.252	−0.824	−0.020
Toe lamellae	−0.257	−0.468	0.402	0.013	−0.344
Finger lamellae	−0.405	−0.389	0.362	0.058	0.132
Variation expl. (79%)	26%	19%	14%	11%	9%

more than 30% and the first five axes (cumulative) still explained less than, but nearly, 80%. For each transect, the characters that on inspection appeared significant (numbers of circumorbitals and femoral pores) loaded heavily on PC1, and PC1 of both the northern and southern transect was loaded most heavily by these two characters. PC1 of the central transect was loaded most heavily by toe lamellae, finger lamellae, circumorbitals, and femoral pores.

Anomalies and symmetry: parents versus hybrids: The morphological characters discussed above involve primarily the descrip-

tions of the two pure taxa (in particular how they differ) and the intermediacy of their hybrids. In addition, we have examined characters to address the question of whether the hybrids have developmental anomalies that may reflect reduced fitness, perhaps through genetic dysfunction. In fact, as primary justification for treating *C. t. marmoratus* as a species separate from *C. tigris*, Hendricks and Dixon (1986: 328) stated that the lizards in the contact region show "developmental asymmetry in head and body scutellation." However, they presented no evidence to support this statement and did not cite any spec-

imens examined from the contact region. Furthermore, we found no evidence to support their statement, either.

The presence of eight longitudinal rows of enlarged ventral plates at midbody is typical for most species of *Cnemidophorus*. Nevertheless, there is variation between and within some taxa, so this should not be assumed to be a fixed character.

We counted the number of ventral rows on hundreds of lizards representing both the pure taxa and the hybrids from the contact region, although many of the specimens could not be counted with precision at midbody due to ventral incisions used to remove tissues. Individuals that differed from the normal condition had atypical fusions (count of 7) or fissions (counts of 9–11).

For pure *C. t. marmoratus*, 8 of 162 specimens (4.9%) deviated from the typical condition of having eight rows of ventrals. For pure *C. t. punctilinealis*, 5 of 90 specimens (5.6%) were atypical. For hybrids, 4 of 103 specimens (3.9%) were atypical. For pure *marmoratus*, specimens from four sites (7, 13, 47, and 48) were all typical, but these were the smallest samples, with only eight or nine specimens examined from each site. The other three sites of pure *marmoratus* varied as follows: site 16, one variant lizard out of 10; site 29, two variants out of 31 lizards; and site 14, five variants out of 88 lizards, or 5.7%. For pure *punctilinealis*, specimens from six sites (1, 10, 11, 21, 22, and 36) were all typical, and these included the smallest samples as well (from 2 to 10 specimens). The other two sites of pure *punctilinealis* varied as follows: site 37, one variant lizard out of 10; and site 20, four variants out of 32 lizards, or 12.5%. Hybrids were examined from nine sites—the midpoint of gene exchange and the site adjacent to each side of it for each of the three transects. Specimens from five sites (4, 27, 40, 42, and 44) were all typical, and these included smaller samples (from 5 to 10 examined from each site). The other four sites of hybrids varied as follows: site 2, one variant lizard out of nine, or 11.1%; site 25, one variant out of 10; site 3, one variant lizard out of 19; and site 26, one variant out of 22. These observations do not suggest that the hybrids are either more

or less variable than the lizards from nonhybrid populations.

Specimens of *Cnemidophorus tigris* typically have the type I condition of the preanal scales, in which two enlarged preanals are side by side anterior to the vent, and a central enlarged one is immediately anterior to these. For seven sites of pure *C. t. marmoratus* with samples ranging from 10 to 88 specimens (sites 7, 13, 14, 16, 29, 47, and 48), deviants from the type I preanal scale character comprised 5.7–50.0% of the sample (overall mean of 10.7%, in a total of 168 specimens). For eight sites of pure *C. t. punctilinealis* with sample sizes ranging from 10 to 32 specimens (sites 1, 10, 11, 20, 21, 22, 36, and 37), deviants from the type I condition of preanals comprised from 0–20.0% (overall mean of 8.8%, in a total of 102 specimens). For nine sites of hybrids (the three centralmost ones of each transect) with sample numbers ranging from 9 to 31 specimens (sites 2, 3, 4, 25, 26, 27, 40, 42, and 44), deviants from the type I preanals comprised from 0–33.3% (overall mean of 12.5%, in a total of 120 specimens). Data for the samples from the midpoint of gene exchange in each transect are as follows: site 3, 10% deviating from type I in 20 specimens; site 26, 9.7% deviating in 31 specimens; and site 42, 0% deviating in 10 specimens. We conclude that there are no significant differences between hybrids and nonhybrids in this character. This is not surprising, as this morphological character has been shown to be variable in a laboratory-reared clone of *Cnemidophorus neomexicanus* (see Dessauer and Cole, 1989), which suggests significant environmental influence on development of preanal scalation.

For additional tests of asymmetry in hybrids versus nonhybrids, we performed paired comparison *t*-tests of the data presented in table 28 for which separate counts were recorded for the left and right sides of the body of each specimen. The following six characters (each a discrete count) were tested for each site: number of circumorbital scales, infralabials, gulars, femoral pores, fourth toe lamellae, and fourth finger lamellae. For the largest samples (sites 14, 20, 26, and 29), of which site 26 is the midpoint of gene exchange for the central transect, we

performed separate tests on males and fe-males, for a total of 48 tests ($6 \times 4 \times 2$). Of these tests, 42 indicated no significant differ-ence ($P > 0.05$) between the left and right sides. The six suggestions of asymmetry are discussed immediately below.

The number of left versus right gulars ap-peared different ($P < 0.05$) in males from site 14. Females were not different for this or any other site. Similarly, the number of left versus right toe lamellae appeared dif-ferent ($P < 0.05$) in males from site 29. However, females were not different for this or any other site. The number of finger la-mellae appeared different ($P < 0.05$) in males from sites 14 and 20 (but no other males) and in females from sites 20 and 29 (but no other females). We regard these tests as reflecting experimental error rather than biological phenomena of special significance, although it is intriguing that 50% of the tests for number of finger lamellae suggested left versus right asymmetry. We are particularly impressed by the fact that none of the sug-gestions of left-side versus right-side asym-metry involved samples from the hybrid pop-ulation, site 26. All involved populations of pure *punctilinealis* or pure *marmoratus*.

Finally, we performed the same paired comparison *t*-tests for the sample from the midpoint of gene exchange of each of the transects of the hybrid zones (sites 3, 26, and 42). We pooled the sexes for these tests be-cause of smaller sample sizes (especially for site 42), but this is acceptable, as we had concluded earlier that there is no sexual di-morphism in these characters (see "Sexual Dimorphism" above). Only one of the 18 tests suggested that there is left-side versus right-side asymmetry ($P < 0.05$; the number of gular scales of lizards from site 3). The means for these characters are very close to each other, however (table 28), so we attach no biological significance to this result. We are more impressed by the lack of asymme-try suggested by the other 17 tests. Our re-sults are consistent with those of other recent studies (Lamb et al., 1990; Patterson and Pat-ton, 1990) that reported literally or essential-ly no differences among hybrids versus non-hybrids in levels of fluctuating asymmetry in natural populations, including a recent study on lizards (Dosselman et al., 1998).

TABLE 30
Egg Production Among Lizards from the Con-tact Region[a]

Sam-ple[b]	Clutch size[c]	Body length[c]	Correlation[d]
PUN	2.4±0.170 (1–4)20	75.5±1.426 (60–89)20	0.49456[e]
HYB	2.8±0.249 (2–5)13	79.4±1.835 (70–91)13	0.62756[e]
MAR	3.0±0.214 (1–5)18	82.2±0.995 (71–92)18	0.35307

[a] Figure 3 is a map of the contact region.

[b] PUN refers to *C. t. punctilinealis,* MAR to *C. t. mar-moratus,* and HYB to hybrids.

[c] Clutch size is number of eggs per clutch; body length is in mm. Data are presented as the mean ± standard error of the mean (observed range) sample size.

[d] This is the correlation coefficient. The relationship between clutch size and body length is shown in figures 47 and 48.

[e] $P < 0.05$.

INTERNAL CHARACTERS (REPRODUCTION AND FITNESS)

The genetic and external morphological data discussed above suggest that panmixia prevails in the contact zones, with lizards of F_1 and subsequent generations being fertile and interbreeding freely. Genotype frequen-cies suggest that all genotypes are equally fit. If this is correct, then there should be no sig-nificant differences observed in the gonads and fecundity of hybrids versus nonhybrids from the contact region. We examined repro-ductive tracts in lizards from each transect to address this question.

We examined testes of 37 pure *C. t. punc-tilinealis* from the three transects (site 1, $N = 6$; site 20, $N = 22$; site 36, $N = 9$). All except one of these were collected between 27 May and 11 July (over several years), and all except one lizard had conspicuously en-larged testes characteristic of actively breed-ing males. The exception was AMNH R-138509 from site 36, which was collected on 23 July 1992, near the end of the breeding season (Parker, 1973; Goldberg and Lowe, 1966). We also examined testes of 24 pure *C. t. marmoratus* from the three transects (site 7, $N = 4$; site 29, $N = 17$; site 48, $N = 3$). These were collected between 23 May
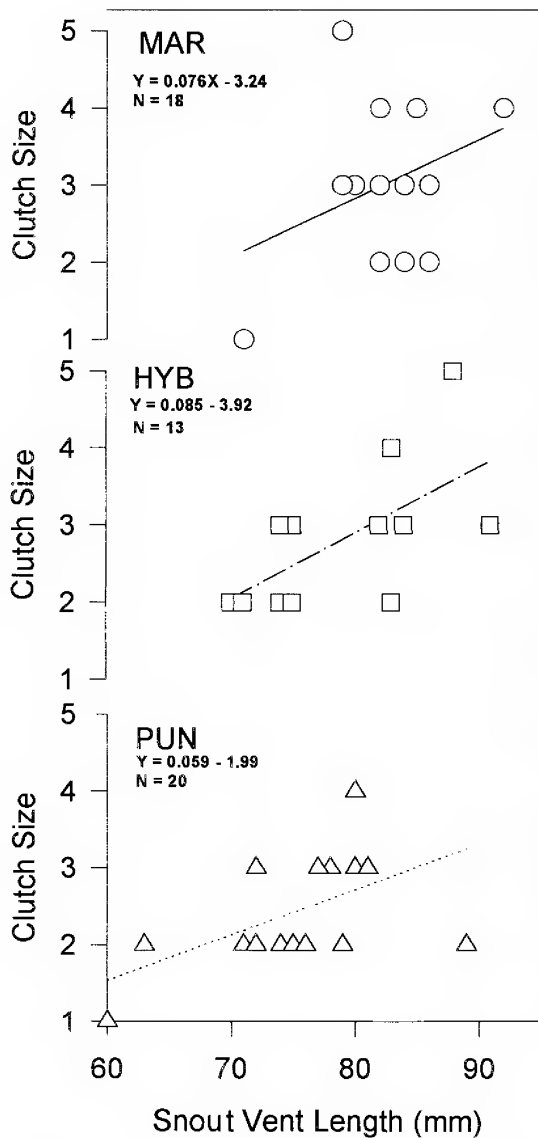
Fig. 47. Relationship between body length and number of eggs per clutch in specimens of *C. tigris* from the contact region. MAR, pure *marmoratus*; PUN, pure *punctilinealis*; HYB, hybrids. Data are summarized in table 30 and figure 48.

Fig. 48. Relationship between body length and number of eggs per clutch (same data as table 30 and fig. 47), showing 95% confidence intervals (broken lines) for each plot. M, pure *marmoratus*; P, pure *punctilinealis*; H, hybrids.

and 9 August (several years), and all but three lizards had enlarged testes. One of the exceptions was AMNH R-139712 (site 29), collected late in the season (22 July 1993), when testes often are regressing. The other two exceptions (AMNH R-127068 and R-127071; site 48) measured 72–74 mm in body length and were the smallest males col-

lected on 7 July 1983 (perhaps being immature—Degenhardt et al. [1996] stated that males reach sexual maturity at about 70 mm body length). We conclude that there is no clear evidence of fertility problems among the pure males of either taxon, and testes are clearly undergoing regression in some individuals by 20 July. However, a series of males from site 48 collected on 9 August 1990 had large testes, which may indicate that this was an exceptional year for breeding in this area.

For hybrid males, we examined testes from the three sites closest to and including the midpoint of gene exchange for each transect. For the northern transect, this included site 2 (6 males), site 3 (11 males), and site 4 (5 males). These were collected between 28 June and 14 July (over several years), and all but one of these 22 lizards had enlarged testes. The exception was AMNH R-136939 (site 4) collected on 29 June with a body length of 76 mm, the

smallest male of that sample. For the central transect we used site 25 (6 males), site 26 (19 males), and site 27 (7 males). These were collected between 21 May and 10 July, and 27 of these 32 lizards had enlarged testes. One of the five exceptions (from site 26) was held in captivity for more than a month and used in physiological fitness experiments prior to dissection (Dohm et al., 1998), and the other four (sites 26 and 27) were collected somewhat late in the season (10–19 July 1993). For the southern transect we used site 40 (6 males), site 42 (7 males), and site 44 (8 males). These were collected between 4 and 12 July 1983, and 18 of these 21 lizards had enlarged testes. The three exceptions were collected on the last days of the series (10–12 July). We conclude that the hybrid males normally do not experience fertility problems.

We determined number of eggs per clutch (clutch size) in 20 females of pure *C. t. punctilinealis* from nine sites in the contact region (site 1, $N = 2$; site 20, $N = 6$; site 36, $N = 1$; site 9, $N = 2$; site 11, $N = 1$; site 21, $N = 2$; site 22, $N = 1$; site 37, $N = 1$; and site 38, $N = 4$). We determined clutch size also in 18 females of pure *C. t. marmoratus* from six sites in the contact region (site 7, $N = 4$; site 29, $N = 4$; site 48, $N = 4$; site 14, $N = 1$; site 16, $N = 2$; and site 47, $N = 3$). In addition, we determined clutch size in the 13 female hybrids from the three sites closest to and including the midpoint of gene exchange in each of the three transects (site 2, $N = 1$; site 3, $N = 3$; site 4, $N = 1$; site 26, $N = 6$; site 27, $N = 1$; site 44, $N = 1$).

The data on clutch sizes are presented in table 30 and figure 47. These data are typical for *C. tigris* from this part of its range (Goldberg and Lowe, 1966; McCoy and Hoddenbach, 1966; Pianka, 1970; Parker, 1972, 1973; Taylor et al., 1994; Degenhardt et al., 1996). Although the mean clutch size observed for *marmoratus* appeared larger than that for *punctilinealis*, the difference was not statistically significant, with the females of *marmoratus* in our samples averaging a bit larger than those of *punctilinealis*, and the hybrids appearing to be intermediate in the means for both clutch size and body length (table 30). Indeed, there is

no significant difference between the taxa or their hybrids in the relationship between body length and clutch size, as results of correlation and regression analyses plotted together with their 95% confidence intervals showed that the three plots were indistinguishable (fig. 48).

Considering that a few apparently nonhybrid lizards may occur at the sites used for the above analyses of hybrids, we reviewed a posteriori the coloration hybrid index data for each of the 13 hybrids analyzed. Scores ranged from 0 to 1.0, with a mean of 0.43. Four lizards were in the range of 0.40–0.60, and nine were in the range of 0.29–0.62. Consequently, there is no indication that the female hybrids are experiencing fertility problems, either. This is to be expected because if there were unequal survivorship in eggs and hatchlings with certain gene combinations, this would have been revealed in the genetic analyses discussed above.

Another way to test for selection against hybrids is to compare fitness in physiological traits that are involved in important activities (e.g., predator escape or foraging). Dohm et al. (1998) compared pure *C. t. punctilinealis* from our site 20 with pure *C. t. marmoratus* from site 29 and hybrids from sites 26 and 3. No significant differences were found for maximum sprint speed, endurance running capacity, standard metabolic rate, blood hematocrit, heart mass, or body mass. The only differences observed in their particular samples were in the weights of livers and kidneys, but the differences in the individuals compared may have been due to differences in reproductive states of specimens in their small samples rather than to genetically based population differences, given the wide intraspecific variation that occurs in these organs (for example, see Dessauer, 1955; Fox, 1958).

In summary, no significant differences have been found in reproductive characters or other measures of fitness among *C. t. punctilinealis*, *C. t. marmoratus*, or their hybrids, as compared in population samples from the contact region. This is consistent with the genetic data on Hardy-Weinberg, linkage, and cytonuclear equilibria (see Biochemical Genetics above).

COMPARISONS WITH THE PAST AND PREDICTIONS FOR THE FUTURE

POSITION OF THE CENTRAL HYBRID ZONE

In an attempt to determine whether the hybrid zone of the central transect had shifted in a quarter of a century, we previously (1991) compared samples with earlier collections of Zweifel (1962) and Dessauer et al. (1962). Owing to color changes on specimens in preservative, only the first three characters of color pattern could be compared with reasonable confidence, including the dorsal stripes, dorsal spots, and lateral spots or bars on the same specimens originally examined by Zweifel (1962). As before, "to make the comparison direct, we recomputed the hybrid index scores on our own nearest samples based on only the same three characters" (Dessauer and Cole, 1991: 634). We concluded that the hybrid zone had either moved eastward about 1 km in 25 years, or that the zone had shrunk on its western side (there were no comparative data for the eastern side).

For the present report we planned to make a similar comparison with specimens from the vicinity of Crystal Mine (site 41). In the process, we discovered that Zweifel (1962: 759) cautioned against this: "Collections made in 1958 were not segregated on such a microgeographic basis, so these specimens cannot be used to support the occurrence of *marmoratus* east of the highway and hybrids to the west."

Nevertheless, we were able to add another sample that improves this comparison across time, and we changed our conclusion as a result. We sampled site 19 (figs. 3 and 5) at 4.8 km (by road) north of Steins in 1991; Zweifel had sampled it in 1960. In this instance, we are quite confident that we sampled precisely the same site as did Zweifel 31 years previously. We scored the mean hybrid index for the first three characters of coloration on Zweifel's six specimens as 0.46 ± 0.094 (range, 0.08–0.67), which indicates the midpoint of gene exchange between *C. t. punctilinealis* and *C. t. marmoratus*. Our sample of 10 lizards from 1991 had a mean of 0.31 ± 0.088 (range, 0.00–0.83). Looking

at the mean only, this is consistent with Dessauer and Cole's (1991) suggestion—lizards at site 19 appear to have gained more influence from *punctilinealis* at the expense of *marmoratus* in the past 30 years. A shift of −0.15 on the coloration hybrid index may represent an eastward shift on the central transect of about 1 km (fig. 31) for the midpoint of gene exchange, consistent with the conclusion of Dessauer and Cole (1991). Since site 19 is north of Steins, approaching the area where the pure populations of *marmoratus* are boxed in near site 16, this is a hybrid zone that could be moving northward from Steins between the east slope of the Peloncillo Mountains and the west side of the alkaline flats and grassland west of Lordsburg (fig. 49, discussed below).

However, this suggestion must be considered quite tentative because a Student's *t*-test shows no significant difference between the means involved ($P > 0.05$). The wide range of variation in coloration at hybrid sites produces such a high variance that often samples from adjacent sites have means with differences that are statistically insignificant, although clear trends exist across the transects (figs. 36–38).

FUTURE OF THE HYBRID ZONES

Figure 49 shows the present distribution of pure *C. t. punctilinealis* (0–0.1 on the coloration hybrid index; table 24) and pure *C. t. marmoratus* (0.8–1.0 on the hybrid index; table 24) in the contact region. Hybrids (0.11–0.79 on the hybrid index; table 24) occur at the sites between the ranges of the pure taxa where hatching on figure 49 is lacking. Note in figure 49 that site 16 represents a population of pure *marmoratus* that is boxed in between the unfavorable habitats of mountains to the west and grassland or playa to the north and east; this population interbreeds with *punctilinealis* to the south. The narrow line with crossbars that runs southeast from site 12 toward Lordsburg in figure 49 represents the railroad tracks (fig. 50) where a fourth hybrid zone presumably with
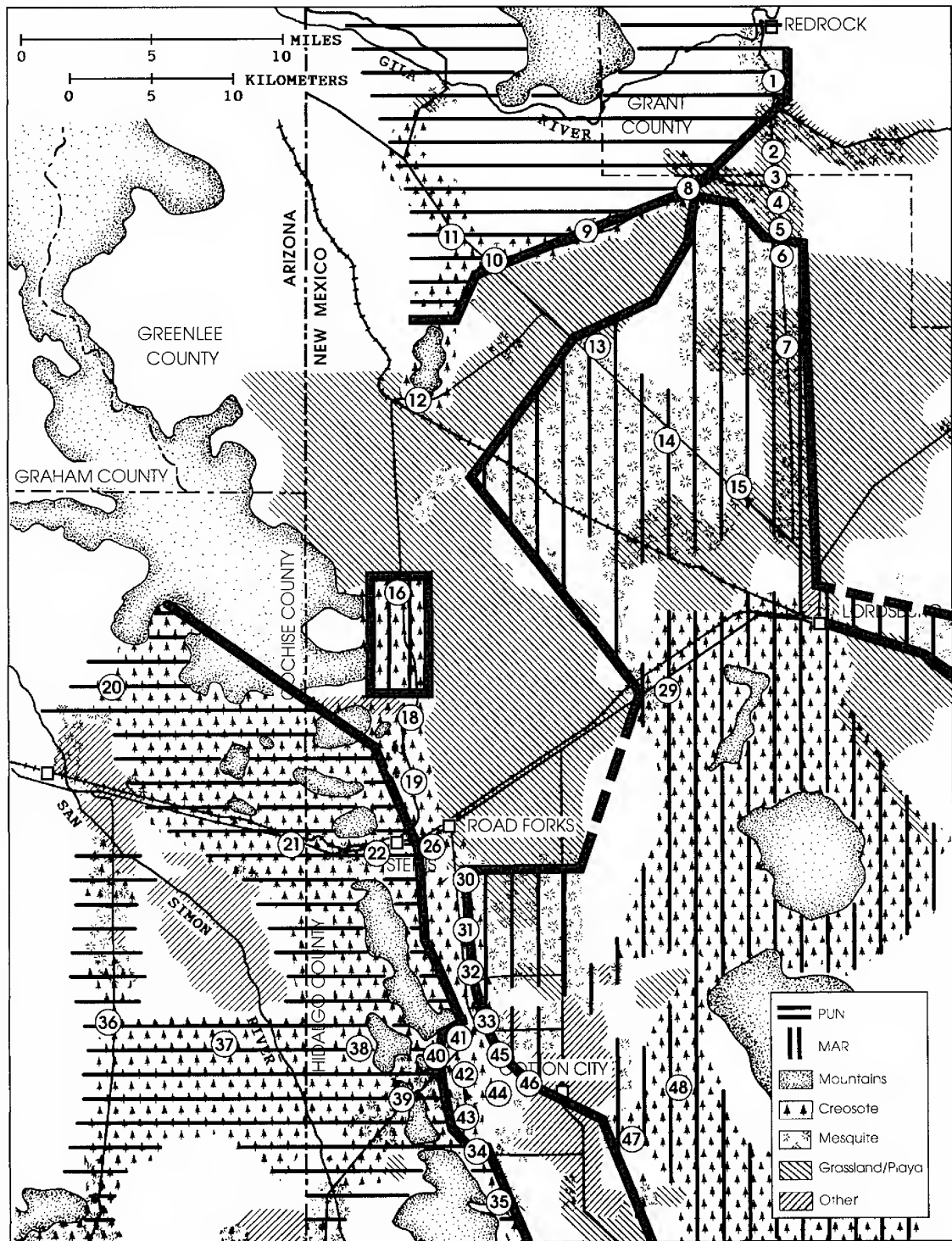
Fig. 49. The contact region. Horizontal lines represent range of pure *punctilinealis* (coloration indices of 0–0.1; table 24), and vertical lines pure *marmoratus* (coloration indices of 0.8–1.0). Sites in between (2–5, 18, 19, 26, and 41–44) represent primarily hybrids (coloration indices of 0.11–0.79).

small populations became apparent as we were completing this study.

During the most recent period of maximum glaciation near the end of the Pleistocene, approximately 18,000 years ago, vegetative communities were quite different in the contact region than they are today, despite the absence of glaciers in the immediate vicinity. In southeastern Arizona and southwestern New Mexico, woodland communities dominated by trees occurred at lower elevations than they do today, Animas Valley and adjacent valleys to the east and southeast contained significant lakes, including Lake Animas and Lake Cabeza de Vaca (Axtell, 1978; Van Devender, 1995), and the Sonoran Desert was displaced to the southwest while the Chihuahuan Desert was displaced to the southeast (Wells, 1966, 1985; Van Devender, 1995; Van Devender et al., 1984). Even if the distance separating these deserts into respective desert refugia was not as great as some specialists have suggested, the Holocene to Recent records of packrat middens analyzed from 18,000 years ago to the present (Van Devender et al., 1984) suggest that populations of *C. t. punctilinealis* and *C. t. marmoratus* may have been separated by unfavorable habitat approximately 5000 years or so before present, perhaps between the longitudes of the Dragoon Mountains, Arizona, and Deming, New Mexico (a distance about twice the width of the contact region; rectangle in fig. 1). This compares favorably with the estimated number of generations during which these taxa may have been interbreeding (see Gene Flow and Age of the Hybrid Zones; table 23). It also seems likely that the genetic differences that now exist between these two taxa evolved through divergence from their common ancestor during an earlier time of geographic separation (allopatry).

More or less coincident with the retreat of the glaciers from higher latitudes, warming and drying trends in the Southwest resulted in expansion of the deserts while tree communities withdrew upslope to higher, cooler, wetter elevations, and Lake Animas and Lake Cabeza de Vaca basically dried up. One credible estimate dates the arrival of creosote (*Larrea*) desertscrub from the southeast to the vicinity of Lordsburg as approximately 3000–4000 years before present, as the Chihuahuan Desert expanded (Van Devender et al., 1984). Assuming that *C. tigris* then occurred in about the same habitats as it does today, it is likely that the easternmost populations of *punctilinealis* occurred somewhere in Arizona to the west of the Peloncillo Mountains, and the low elevation passes of the Peloncillos contained habitat unfavorable to *C. tigris*—numerous junipers (*Juniperus*) and oaks (*Quercus*), if not also pines (*Pinus*)—until around 3000 years ago.

As Lake Animas and other lakes nearby dried up, the woodlands retreated to higher elevations while the Sonoran Desert expanded to the east and the Chihuahuan Desert expanded to the west, along with their respective faunas. Finally, desertscrub corridors emerged through the low-elevation passes in the Peloncillo Mountains. The barrier of unfavorable habitat that previously separated *C. t. punctilinealis* and *C. t. marmoratus* was replaced by their preferred habitat, and secondary contact was made. Contact in the northern hybrid zone probably involved populations of *C. t. punctilinealis* that dispersed eastward along the Gila River around the northern end of the Peloncillo Mountains, whereas contact in the central and southern hybrid zones involved *punctilinealis* that followed the San Simon Valley southeastward from the Gila River on the west side of the Peloncillo Mountains.

While the paleontological data suggest that the most recent contact between *punctilinealis* and *marmoratus* occurred perhaps within the last 3000 years, historical records with repeat photography (fig. 2; Hastings and Turner, 1965; Humphrey, 1987; Bahre, 1991, 1995; Bahre and Shelton, 1993) illustrate that desertification has continued in places, with significant invasion of grasslands by woody shrubs (including *Flourensia*, *Larrea*, and *Prosopis*), even during the last 130 years. Given this background, a review of habitat photographs showing juniper trees beside the desertscrub habitats within the narrow low-elevation corridors in the Peloncillo Mountains (figs. 20, bottom, 21, and 22, top) suggests that the most recent uninhibited and continuous contact of these lizards could have occurred on the order of hundreds of years ago rather than thousands (table 23),

although we do not know for sure. Also, there could have been some infrequent contact and interbreeding between these populations based on random dispersal prior to establishment of continuous corridors of contact.

At any rate, plant communities within the contact region are probably still shifting, and these shifts can be expected to affect future distributions of the lizards and the hybrid zones. This is the main reason why we have tried to pinpoint so precisely what is happening today and where; a reanalysis of strategically selected sample sites used for this study, perhaps to be conducted 100 years or more after publication of this report, should be informative. We hope that the genetic data, habitat maps and photographs, descriptions, and detailed site data provided here will constitute useful baseline information for developing improved understanding of the underlying processes. Future researchers need not resample every site, but they can use our data to develop an efficient resampling strategy.

One paradox of special interest concerns the genetic analyses discussed above and the morphological data discussed in the context of a hybrid zone that may be moving. The genetic analyses (particularly allele frequencies, linkage and cytonuclear equilibrium, and concordant and coincident clines having widths consistent with age of contact) are all consistent with a scenario of neutral secondary contact involving slow intergradation of alleles. This would predict stability in geographic location of the center of the cline (Barton and Hewitt, 1985) coincident with widening of the zone of intergradation. Alternatively, narrow step-clines that are shifting in geographic location in the vicinity of population density sinks are usually "tension zones," which involve selection or unequal immigration (Barton and Hewitt, 1985), for neither of which we have evidence.

While it can be relatively easy to generate scenarios explaining matters of evolutionary and ecological history, biological reality can be elusive and considerably more complex than scenarios suggest. For example, while we have been focusing so far on the most recent contact between *C. t. punctilinealis* and *C. t. marmoratus*, this might not be the first and only one. As discussed by Dessauer and Cole (1991), there may have been several periods of contact and loss of contact between these populations or their ancestors during many of the alternating 15–20 interglacial and glacial episodes that occurred during the 2.4 million years of the Pleistocene (Van Devender, 1995). In addition, while the general warming and drying trend progressed through the Holocene–Recent, there probably were significant episodes of weather reversals and other fluctuations from time to time that may have affected the contact of these populations in relatively sensitive or fragile arid and semiarid ecosystems. In addition to these relatively recent events, in the more distant past, prior to the divergence of *punctilinealis* and *marmoratus* from their desert-adapted common ancestor, that ancestor may have dispersed through the same corridors, after which the modern taxa would have evolved in small, widely separated desert refugia on opposite sides of the Sierra Madre Occidental after the corridors were closed, perhaps during the first significant period of Pleistocene glaciation. Different characters of the modern lizards probably reflect genetic traces of these different events through time, as was discussed by Patton and Smith (1994) for pocket gophers.

While conducting the final review of our genetic data for this manuscript, we realized that we found more alleles characteristic of *C. t. marmoratus* than we had expected at site 12, in typical *punctilinealis* territory to the northwest of the grassland that separates these taxa northwest of Lordsburg (in particular, compare frequencies of the a-allele at TF and the M mtDNA at sites 10 and 12; table 7). This increased frequency was more than would be consistent with gene flow southwestward from site 8 through sites 9 and 10 (fig. 49). Instead, it appeared as if there was leakage of *marmoratus* alleles across the grassland northwest of Lordsburg, perhaps along the railroad tracks (fig. 49). Consequently, on 3 August 1997 we walked from site 12 for a mile or more to the southeast toward Lordsburg, along the railroad tracks. There are patches of *Prosopis* and *Larrea* scattered through this area and also a thin scattering of these shrubs right along the railroad tracks (fig. 50). Some of these shrubs

Fig. 50.   Grassland WNW of Lordsburg, looking to the NW toward the Summit Hills that are immediately north of site 12 (fig. 49). Dark band of vegetation at the base of the hills is the creosote community. Thin diagonal line across grassland is the railroad, with shrubs along the tracks. Aerial photograph taken on 1 September 1990.

are the largest mesquites we have seen in the immediate area, and we wonder whether they benefit from moisture retention provided by the track bed. It even seems likely that microhabitat disturbance stemming from construction of the railroad may have inadvertently increased the shrub distribution across this stretch. Because density of the shrubs varies and in places is thin, there must be a population density sink among the *C. tigris* here, but nevertheless there probably are some living here. This probably is a fourth hybrid zone that could be investigated in the future. The area is accessible, as there is a dirt road along the north side of the railroad tracks, which probably can be reached from the railroad crossing at Summit, about 1 km west of site 12 (fig. 49).

COMPARISONS WITH ADDITIONAL SUBSPECIES OF *C. TIGRIS*

Table 31 lists the genotypes we found in population samples (fig. 1; append. 2) of two additional adjacent subspecies, *C. tigris aethiops* and *C. tigris septentrionalis*. Comparing these data with tables 6–12, we identify those gene loci that are most useful for distinguishing among these taxa (table 32). The alleles shared at most other loci are more conservative and occurred in more distant common ancestors, some being conservative for ancient phylogenetic links between *Cnemidophorus* and other genera (for example, HB, LDH1, and sSOD; see Dessauer and Cole, 1989).

A comparison of all tables of genotypes (tables 6–12 and 31) for populations within the contact region or distant to it suggests that *C. t. marmoratus* is not as genetically distinctive among all populations of *C. tigris* as one might expect after examining the sharp step-clines among samples from the contact region. In fact, if subspecies are to be recognized at all, the genetic similarities shared in this complex are consistent with recognizing all of these taxa as subspecies of one species, *C. tigris*.

At sMDHP, the a-allele of *marmoratus* was essentially absent from *punctilinealis* in the contact region (but see sites 36 and 37 of the southern transect; table 10), yet the a-

TABLE 31

Frequencies of Genotypes at Collecting Sites for *C. t. septentrionalis* and *C. t. aethiops*[a]

Locus[b]	57 (11)	58 (3)	59 (5)	60 (2)	61 (7)	62 (1)	63 (2)	64 (3)
	septentrionalis						*aethiops*	
Oxidoreductases								
ADH								
aa	11	3	5	—	7	1	—	—
bb	—	—	—	2	—	—	—	—
cc	—	—	—	—	—	—	2	1
G3PDH								
aa	11	3	5	2	7	1	2	1
IDDH								
bb	11	3	5	2	7	1	2	1
LDH1								
aa	11	3	5	2	7	1	2	1
LDH2								
bb	11	3	5	2	7	1	2	1
sMDH								
ab	—	—	—	—	—	—	—	1
bb	11	3	5	2	7	1	2	—
mMDH								
bb	11	3	5	2	7	1	2	1
sMDHP								
aa	3	3	—	2	2	—	1	1
ab	2	—	—	—	—	—	—	—
bc	5	—	—	—	—	—	—	—
sIDH								
ab	1	—	—	—	—	—	—	—
bb	10	3	5	2	7	1	2	1
mIDH								
aa	11	3	5	2	3	1	2	1
sSOD								
aa	11	3	5	2	7	1	2	1
mSOD								
aa	11	3	5	2	7	1	2	1
DDH								
aa	11	3	5	2	7	1	2	1
Transferases								
sAAT								
bb	11	3	5	2	7	1	—	1
bc	—	—	—	—	—	—	2	—
mAAT								
bb	11	3	—	2	7	1	2	1
AK								
aa	11	3	5	2	7	1	2	1
CK1								
bb	11	3	5	2	7	1	2	1
CK2								
aa	11	3	5	2	7	1	2	1
Hydrolases								
ALP								
aa	11	2	5	2	6	1	2	1

TABLE 31—(*Continued*)

Locus[b]	57 (11)	58 (3)	59 (5)	60 (2)	61 (7)	62 (1)	63 (2)	64 (3)
	septentrionalis						*aethiops*	
EST1								
bb	1	3	—	1	1	—	2	1
EST2								
aa	—	1	—	—	—	—	2	1
ab	1	—	1	2	1	1	—	—
bb	10	2	1	—	2	—	—	—
ESTD								
aa	—	—	1	1	1	—	—	—
ab	2	1	1	1	4	—	—	—
bb	9	2	3	—	2	1	2	1
PEPA								
bb	11	3	5	2	7	1	2	1
PEPB								
ab	2	—	—	—	—	—	—	—
bb	9	3	5	2	7	1	—	1
bc	—	—	—	—	—	—	2	—
PEPD								
aa	3	—	—	—	—	—	—	—
ab	—	—	—	—	—	—	1	—
bb	—	—	—	—	—	—	1	1
ac	7	1	—	2	—	—	—	—
ae	—	—	—	—	1	—	—	—
bc	—	—	2	—	2	—	—	—
bd	—	—	—	—	—	1	—	—
be	—	—	—	—	1	—	—	—
cc	—	2	3	—	3	—	—	—
ce	1	—	—	—	—	—	—	—
PEPE								
aa	11	3	—	2	7	1	2	—
ADA								
bb	7	2	1	—	4	—	2	1
bc	2	—	4	1	1	—	—	—
cc	2	1	—	1	2	1	—	—
Lyases								
sACOH								
ab	1	—	—	—	—	—	—	1
bb	10	3	5	2	7	1	2	—
mACOH								
aa	3	2	—	—	7	1	2	1
ab	—	1	—	—	—	—	—	—
Isomerases								
MPI								
bb	11	3	5	2	7	1	2	1
GPI								
aa	—	—	3	2	5	1	—	—
ab	7	—	1	—	2	—	—	—
bb	4	3	—	—	—	—	2	1
bc	—	—	1	—	—	—	—	—

TABLE 31—(*Continued*)

Locus[b]	Site[c]							
	septentrionalis						*aethiops*	
	57 (11)	58 (3)	59 (5)	60 (2)	61 (7)	62 (1)	63 (2)	64 (3)
PGM1								
aa	—	—	—	—	—	—	2	1
cc	11	3	—	2	7	1	—	—
PGM2								
bc	—	1	—	—	—	—	—	—
cc	11	2	5	2	7	1	—	—
dd	—	—	—	—	—	—	2	1
PGM3								
aa	3	—	4	1	5	1	—	—
ac	4	1	1	1	2	—	—	—
bb	—	—	—	—	—	—	2	1
cc	4	2	—	—	—	—	—	—
Nonenzymic Blood Proteins								
TF								
bb	11	3	5	2	6	1	2	3
bc	—	—	—	—	1	—	—	—
HB								
aa	11	3	5	2	7	1	2	1

[a] Collecting sites and subspecies are described in appendix 2 and mapped in figure 1.

[b] Locus abbreviations are defined in table 5. Alleles for protein loci are designated in alphabetical sequence in order of decreasing anodal migration.

[c] Numbers of lizards studied are listed in parentheses. Where the number of observations within columns do not total the number of lizards in the sample, data were missing for one or more individuals.

allele appeared to be in *aethiops, septentrionalis*, and *punctilinealis* specifically from sites 49 and 56 (table 12). At TF, the a-allele of *marmoratus* was essentially absent from *punctilinealis* in the contact region (but see

site 20 of the central transect; table 8), yet the a-allele appeared to be in some *punctilinealis* from site 49 (table 12). In fact, only two alleles appeared to be totally unique to *C. t. marmoratus*, but neither of these was genetically fixed, as follows: (1) the a-allele of IDDH (but the b-allele more common in *marmoratus* was shared with all of the other subspecies; table 32), and (2) the d-allele of PEPB (but the b- and c-alleles more common in *marmoratus* were shared with most of the other subspecies; table 32). Note that the rare c-allele at ADA in *punctilinealis* (table 6) apparently is fairly common in *septentrionalis* (table 31).

In addition to those alleles just mentioned and the rare alleles discussed above (see Uncommon Alleles above), the following seven alleles appear to be unique among the taxa examined in this study (table 32): (1) the c-allele at ADH in *aethiops*; (2) the c-allele at sMDHP in *septentrionalis*, which also shares the a- and b-alleles with the other taxa; (3) the c-allele at EST2 in *punctilinealis* from site 49, which also shares the a- and b-alleles with the other taxa; (4) the c-allele at PGM1 in *septentrionalis*; (5 and 6) the c- and b-alleles at PGM2 in *septentrionalis* (fig. 51); and (7) the c-allele at TF in *septentrionalis* from site 61, which shares the b-allele with most of the other taxa. Given these patterns of local polymorphism and geographic variation, the genetic distinctions among these taxa are not very great.

A glance at the geographic distributions (fig. 1) and the concordant and coincident step-clines in genetic and morphological characters in the contact region (figs. 39–43)

TABLE 32
Alleles that Distinguish Subspecies of *Cnemidophorus tigris*

Subspecies	Loci								
	ADH	IDDH	sMDHP	EST2	ESTD	PEPB	PGM1	PGM2	TF
aethiops	c	b	a	a	b	b, c	a	d	b
punctilinealis (Tucson)	a, b	b	a>b	a, b, c	b	a, b, c	a	d≫a	b≫a
punctilinealis (contact)	a	b	b	b≫a	b≫a	b>c	a	d≫a	b
marmoratus	a	b>a	a≫b	a≫b	b≫a	c>b, d	a	d≫a	a
septentrionalis	a≫b	b	a, b, c	b>a	a, b	b≫a	c[a]	c≫b	b≫c[b]

[a] The c-allele of PGM1 may have the same mobility as the c-allele of PGM2.

[b] One bc heterozygote was observed in a sample of seven lizards from 3.6 miles east of Bluff, Utah. Use of ≫ indicates that the second allele was of rather low frequency.
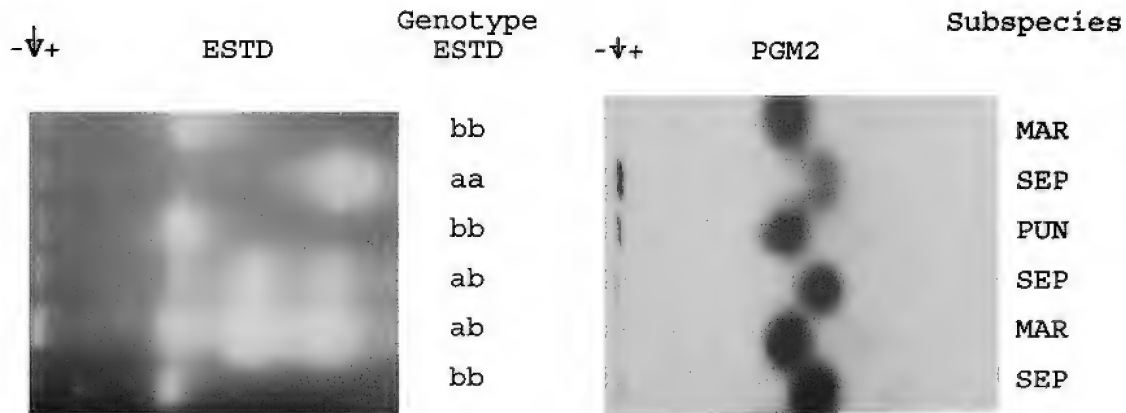
Fig. 51. Electrophoretic phenotypes of proteins of several subspecies of *C. tigris*. **Left**. ESTD polymorphism in *C. t. septentrionalis*. The fluorescent patterns were photographed in ultraviolet light. **Right**. Banding patterns of PGM2 that distinguish *septentrionalis* (SEP, genotype cc) from *punctilinealis* (PUN, genotype dd), *marmoratus* (MAR, genotype dd), and *aethiops* (genotype dd, not illustrated). Arrows indicate sites of sample application; anode is to the right.

might lead one to conclude, perhaps incorrectly, that all of the local differences between *C. t. punctilinealis* and *C. t. marmoratus* evolved specifically between these taxa while they were last isolated in separate desert refugia. However, a review of the genetic characters involved suggests that this view is too simplistic. We now discuss each of the loci represented in these step-clines (figs. 30–32).

IDDH: All taxa share the b-allele (table 32), but the a-allele does fit the scenario of having had a unique origin in *marmoratus*, although its frequency is less than or about 0.50 in most populations of *marmoratus* sampled, including those distant from the contact region, to the east along the Rio Grande (the highest frequency was 0.65 at site 13).

sMDHP: The b-allele was found in *punctilinealis* and rarely in *marmoratus* in the contact region, as well as in the other taxa, excluding *aethiops* (our smallest sample, with five specimens analyzed for proteins). The a-allele characterizes the *marmoratus* in the contact region and is absent from *punctilinealis* there. Elsewhere, however, the a-allele does occur in *aethiops*, *septentrionalis*, and *punctilinealis* (sites 49 and 56).

EST2: The b-allele is typical of *punctilinealis* in the contact region, whereas the a-allele typifies *marmoratus* there. However,

both alleles are found in *punctilinealis* from site 49, both occur in *septentrionalis*, and the a-allele occurs in *aethiops* also.

PEPB: The b-allele that typifies *punctilinealis* in the contact region occurs in all four taxa. The c-allele that is most frequent in *marmoratus* occurs also in all of the others except *septentrionalis*. The d-allele does appear to be unique to *marmoratus*, but it is relatively rare in the contact region and was not found in *marmoratus* from sites 53 and 54 to the east. If there is any validity to the hypothesis that rare alleles are spawned in hybrid zones, it may be possible that this applies to the d-allele at PEPB, but most of our data do not support this hypothesis (see "Uncommon Alleles" above).

TF: The b-allele that characterizes *punctilinealis* in the contact region occurs in all of the other taxa except *marmoratus*. The a-allele that characterizes *marmoratus* was found also in a specimen of *punctilinealis* from site 49, south–southeast of Tucson.

mtDNA: Haplotype diversity was not determined for each subspecies. We analyzed this in *punctilinealis* and *marmoratus* only, and found three types among them (tables 6–12). One type was unique to *marmoratus* and occurred in all of them (plus hybrids); one type was unique to *punctilinealis* from the contact region and occurred in all of them (plus hybrids); and the third type occurred in

all *punctilinealis* from sites to the west of the contact region (sites 49 and 56). Frequencies of the two haplotypes of mtDNA in the hybrid zones were consistent with the hypothesis that females of both types were equally involved in hybridization and panmixia prevails.

It is possible that there is experimental error in recording data from the gels. For example, the *marmoratus*-like a-allele at the TF locus that we recorded for a *punctilinealis* from site 49 might have been a variant from a different allele that encoded a polypeptide with the same charge density as that encoded by the a-allele, thus masquerading as it. However, our interpretation was checked carefully with repeat runs, and it is parsimonious. This allele may be lingering genetic evidence of an earlier episode of contact and interbreeding between *punctilinealis* and *marmoratus*, from which a few genetic traces remain distant from the present contact region; it could be alleles lingering from an ancient ancestral polymorphism; or it could be from Recent introgression. A review of the genotype data (tables 6–12), in concert with the maps of the contact region (figs. 3–5), suggests that some genes typical of each form do spread into populations of the opposite form away from the areas of the most dynamic gene exchange in the center of the current hybrid zones.

Consider first the northern transect (figs. 4 and 49) and associated sites (tables 6 and 7). One *marmoratus* a-allele of TF was found among the *punctilinealis* from site 1, and two *punctilinealis* mtDNAs were found among the nearly pure *marmoratus* from site 6, although none was found at site 5. A nearly 50:50 allele frequency and morphological hybrid index at site 8 leads to a progressive increase in *punctilinealis* characters from sites 9 to 11, probably representing gene flow associated with the contact at site 8; note that site 13, directly across the unfavorable grassland habitat from site 10, consists of typical *marmoratus*. However, the large sample of typical *marmoratus* from site 14 had three *punctilinealis* alleles at TF (2.1%). We do not know from which direction (north, north-

west, or west–northwest) these *punctilinealis* alleles introgressed into the *marmoratus* at site 14. It is also intriguing that some *marmoratus* alleles at IDDH and TF occur in the largely *punctilinealis* at site 12, perhaps indicating gene leakage in disturbed habitat along the railroad tracks (see Comparisons With the Past and Predictions for the Future above).

Consider next the central transect (figs. 5 and 49) and associated sites (tables 8 and 9). One *marmoratus* TF a-allele was found in the large sample of *punctilinealis* from as far west as site 20 ($N = 31$ lizards), but none was found in the sample from site 21 ($N = 10$).

Consider finally the southern transect (figs. 5 and 49) and associated sites (tables 10 and 11). One *punctilinealis* mtDNA was found in a *marmoratus* from the eastern site 48, although none was found at sites 47, 46, or 45 to the west. This *punctilinealis* haplotype probably introgressed eastward across the relatively unfavorable habitat in the floor of the Animas Valley north of Animas. The *marmoratus* sMDHP was found all the way west to *punctilinealis* sites 36 and 37, although it was not found at site 38. This *marmoratus* allele probably introgressed westward across the San Simon River. Finally, the *marmoratus* sMDHP alleles found in the *punctilinealis* from site 51 in Antelope Pass of the Peloncillo Mountains to the south of our map of the contact region (fig. 3) may reflect a new contact zone currently forming. Alternatively, these may be relictual *marmoratus* characters that have been all but swamped out there, west of Animas.

In summary, despite the abrupt clarity of the step-clines in the transects, there are examples of gene leakage or introgression occurring as well. Past events of gene transfer may be reflected in some of the unexpected alleles found at sites distant from the contact region (for example, site 49), or some of these alleles may be lingering from polymorphisms that originally occurred in an ancient common ancestor.

TAXONOMIC STATUS OF *C. T. MARMORATUS*

The taxonomic status of *C. t. marmoratus* was discussed most recently by Dessauer and Cole (1991), who retained *marmoratus* as a subspecies of *C. tigris*. All of the data presented here, including new data pertaining to linkage equilibrium, gene flow, and comparative fitness, are consistent with that conclusion, unless one has a philosophy of not recognizing subspecies. Equally consistent is the taxonomic treatment of additional subspecies of *C. tigris* among which interbreeding has been analyzed by Taylor (1988; *C. t. tigris* vs. *C. t. septentrionalis*), Taylor (1990) and Taylor et al. (1994; *C. t. tigris* vs. *C. t. punctilinealis*), and Taylor and Walker (1991; *C. t. punctilinealis* vs. *C. t. aethiops*). In addition, treating these taxa as subspecies is consistent with the newest phylogenetic analysis of bisexual forms of *Cnemidophorus*, which indicates that all of these forms are within a monophyletic group (Reeder et al., in prep.).

It is beyond the scope of this paper to review all aspects of the philosophy and practice of whether one should recognize subspecies. Instead, we comment on six basic issues that taxonomists with alternative perspectives may find worthy of discussion today.

1. Conspicuous Characters Can Be Used to Diagnose Species: On the basis of coloration, *punctilinealis* and *marmoratus* are diagnosable throughout the vast majority of their ranges, so they could be treated as separate species. However, a very large number of individuals from the contact region could not be identified to species based on morphological characters (table 27). Such individuals are not rare or unusual, but they represent the norm in hybrid zones, where intergradation is apparently under way and nonassortative mating prevails.

2. Characters That Are Fixed in Certain Populations Should Be Used to Diagnose Species, Regardless of How Cryptic They May Be: The a-allele at TF is fixed in all pure populations of *marmoratus*. However, it appeared in one individual of *punctilinealis* from west of the contact region (site 49), and it occurs in numerous heterozygotes within the contact region. Similarly, the mtDNA haplotype that characterized all individuals of *punctilinealis* from the westernmost sites (49 and 56) was unique, and it could be used to diagnose these populations as different from the *punctilinealis* of the contact region. However, specimens for which the haplotype of mtDNA has not been studied could not be identified, and there may be places where both haplotypes (and others) occur. No other characters suggest that the specimens from sites 49 and 56 represent another species.

3. Populations That Differ Should Not Be Treated as Conspecific Because Differences Reflect History and Relationships: On a more fine-grained scale, there are many characters with a unique and interesting history that merit study although they are not applied taxonomically. Examples include alleles involved with hemophilia, blood groups, histocompatibility, and G6PDH levels, as studied in human beings, as well as various genetic characters in lizards. Analyses of certain lineages can illuminate aspects of history, but that does not mean that lineages and species are the same thing.

4. Steep Step-Clines Indicate Interspecific Hybridization: When steep step-clines are caused by selection against hybrids, this is a strong argument for concluding that the hybridizing taxa are different species. However, for the contact region discussed here, there is no evidence for selection against hybrids, and width of the steep step-clines is consistent with the amount of gene flow expected for a model of neutral secondary contact in a timeframe consistent with the most recent reconnection of the Sonoran and Chihuahuan Deserts (see Gene Flow and Age of the Hybrid Zones above). Although steeper than the morphological clines between *C. t. tigris* and *C. t. septentrionalis* (Taylor, 1988) and between *C. t. tigris* and *C. t. punctilinealis* (Taylor, 1990; Taylor et al., 1994), the only impediment to more extensive intergradation in this contact region has been, and continues to be, time.

5. Subspecies Are Not Necessarily Incipient Species: This is true, and, contrary to some opinions, most systematists do not mean to imply that they are. In this instance, we find the concept useful for identifying significantly differentiated populations that

are not on separate evolutionary tracks, even though they may or may not become so in the future.

6. We Cannot Predict the Future Outcome of Interbreeding: The evidence presented in this paper is most consistent with concluding that as sure as there will be lizards breeding next season, the clines in the contact region will be widening. What we cannot predict is whether a future investigator will find credible evidence for subtle selection against hybrids that we have been unable to detect with our samples and methods.

In summary, our view on the use of subspecies may be seen as a blend of the views expressed by Barrowclough (1982; although today he personally applies these opinions toward phylogenetic species rather than subspecies), Avise and Ball (1990), Patton and Smith (1994), Taylor et al. (1994), and Wake and Schneider (1998) in the context of understanding geographic variation and evolutionary processes from the perspectives of population genetics and time. Because of the random mating between *C. t. punctilinealis* and *C. t. marmoratus* in the contact region, as well as the apparently equal fitness of individuals with the various genotypes, these two taxa within a monophyletic group do not appear to be on irretrievably independent evolutionary tracks. Analyses of Hardy-Weinberg equilibrium, linkage equilibrium, *F* statistics, cytonuclear equilibrium, reproduction, and physiological fitness parameters all fail to indicate that individuals with certain genotypes are less fit than others. Using a model of neutral secondary contact, widths of the step-clines are consistent with expectations based on recency of contact and a low level of gene flow owing to the low vagility of *C. tigris*, but with unobstructed intergradation of alleles. With respect to the processes affecting random mating, survival, reproduction, fitness, and gene flow, there are no apparent differences between populations within the hybrid zones and populations outside them, even considering local polymorphisms.

We conclude that the process of allopatric speciation was not completed prior to the reestablishment of contact between these populations, and today they have a parapatric distribution with free gene exchange at each point of contact. The situation seems no different from that of the locally differentiated populations of human beings prior to the modern era of transportation and geographic dislocation of many people. The genetic phenomena would be the same if we artificially transported and intermixed different geographic samples of *C. tigris*.

INTERSPECIFIC HYBRIDIZATION (*C. TIGRIS* × *C.* SPP.)

ORIGINS OF UNISEXUAL SPECIES

Individuals of *Cnemidophorus tigris* occasionally interbreed with lizards of other species, including both bisexual and unisexual species (for reviews, see Dessauer and Cole, 1989; Wright, 1993; and Cole and Dessauer, 1995). The diploid unisexual *Cnemidophorus neomexicanus* originated through cloning of an F$_1$ hybrid female of *C. t. marmoratus* ♀ × *C. inornatus* ♂ (Lowe and Wright, 1966; Brown and Wright, 1979; Cole et al., 1988; Densmore et al., 1989; Dessauer et al., 1996b). The diploid unisexual forms of the *Cnemidophorus tesselatus* complex (including *C. dixoni*) originated through cloning of an F$_1$ hybrid female of *C. t. marmoratus* ♀ × *C. septemvittatus* (or *C. gularis septemvittatus*) ♂ (Wright and Lowe, 1967; Brown and Wright, 1979; Densmore et al., 1989; Dessauer et al., 1996b; see Walker et al. [1997] for use of the name *C. tesselatus* for the diploids in this complex and *C. neotesselatus* for the triploids).

The hybridization mentioned above resulted in unisexual clones that perpetuate the F$_1$ hybrid state parthenogenetically. However, the hybrids produced have been rare, and today the parental taxa remain in sympatry at some localities, without evidence of continued interbreeding.

In addition, tetraploid hybrids have been produced in nature by interbreeding between the triploid, unisexual, clonal *Cnemidophorus sonorae* ♀ × *C. t. punctilinealis* ♂, which may have been sterile (Lowe et al., 1970a), and a sterile tetraploid hybrid was

produced in the laboratory by interbreeding between *C. sonorae* ♀ × *C. t. marmoratus* ♂ (Cole, 1979; Hardy and Cole, 1998). The high frequency of hybridization among taxa of *Cnemidophorus* suggests that there is relatively little discrimination in mate selection among these lizards. This is consistent with the high number of unisexual clones of hybrid origin in this genus and with the panmixia occurring in the hybrid zones described here.

SEX RATIOS IN THE CONTACT REGION

Considering that all-female (unisexual) species of *Cnemidophorus* are of hybrid origin, it is of special interest to examine frequencies of the sexes in the contact region to question whether there is a preponderance of females. This would be expected if an all-female clone of hybrid origin was included among these specimens of *C. t. punctilinealis* × *C. t. marmoratus*.

Appendix 3 lists the sex of the specimens from the sites in the contact region. We summarized the sex ratio data for seven sites of pure *punctilinealis* (sites 1, 10, 11, 20, 21, 36, and 37), for five sites of pure *marmoratus* (sites 7, 13, 14, 16, and 29), and for 10 sites of hybrids, including the midpoint of gene exchange for each transect and two adjacent sites to each side of it (sites 2, 3, 4, 19, 25, 26, 27, 40, 42, and 44). For 19 of these 22 sites (total), males outnumbered females, which is not unreasonable, as most of our collecting apparently was done at times when females were inactive (i.e., preparing for oviposition) and males were feeding and seeking mates. For two sites (4 and 10) there were five of each sex. The only site that produced more females than males was site 16, which consisted of pure *marmoratus*. Totals for these sites were as follows: *punctilinealis*, 29 females and 62 males; *marmoratus*, 62 females and 81 males; hybrids, 49 females and 81 males. We conclude that there is no preponderance of females among these hybrids and that reproduction of *Cnemidophorus tigris* in the contact region involves strictly Mendelian patterns of inheritance, consistent with the genetic data described

above (see "Biochemical Genetics"), with very few exceptions (see below).

HYBRIDIZATION WITH THE UNISEXUAL *C. NEOMEXICANUS*

While electrophoretically screening the genotypes of more than 600 lizards identified in the field as *C. tigris* from the contact region, we noticed that two individuals had genotypes clearly characteristic of polyploids. For example, these lizards had three different alleles functional simultaneously at PEPA. One of these (AMNH R-136962) was among a sample of 10 lizards from site 2, and one (AMNH R-137669) was among 11 lizards from site 10. We did not include these two individuals in appendix 3 because they proved not to be *C. tigris*. However, we had not questioned the identity of these two animals in the field, as they appeared similar to *tigris*, particularly considering the morphological variance in the hybrid zones.

We addressed three working hypotheses for what these unusual individuals could be: (1) hybrids between *C. uniparens* × *C. tigris*, as *uniparens* had been observed at the same collecting sites; (2) triploid backcross hybrids of (*C. t. punctilinealis* × *C. t. marmoratus*) × *C. tigris*, which would have suggested the possible fertilization of an egg cloned from within *C. tigris*; and (3) hybrids between *C. neomexicanus* × *C. tigris*, as *neomexicanus* has been observed at these sites also. If hybridization had involved a maternal *C. uniparens* (unisexual, triploid), the unusual individuals would have been tetraploids, as in the hybrids with *C. sonorae* (see Cole, 1979; Hardy and Cole, 1998). If it involved a maternal *C. neomexicanus* (unisexual, diploid), the unusual individuals would have been triploids, as gene dosage indicated they were (Neaves and Gerald, 1969; Dessauer and Cole, 1984). In any event, they were polyploids of a kind never seen before.

Fortunately, the distinctive genotypes revealed by protein electrophoresis allowed us to distinguish among all of these possibilities (table 33). The possibility that *C. uniparens* was one of the parents of either of these unusual specimens was ruled out by genotypes at 12 loci: they lack the b-allele at ADH, lack

TABLE 33
**Genotypes of Two Triploid Hybrids (HYB) and Two Unisexual Species, *Cnemidophorus
neomexicanus* (NEO) and *C. uniparens* (UNI)
(Plus alleles in samples of *C. tigris* (PUN) from sites 2 and 10 in the contact region[a])**

Locus[c]	Genotypes				Alleles	
	UNI (3n[b])	NEO (2n)	HYB (3n) (site 2)	HYB (3n) (site 10)	PUN (2n) (site 2)	PUN (2n) (site 10)
ADH	bcc	ac	aac	aac	a	a
G3PDH	aaa	aa	aaa	aaa	a	a
IDDH	abb	bb	bbb	bbb	b>a	b
LDH1	bbb	ab	aab	aab	a	a
LDH2	bbb	bb	bbb	bbb	b	b
sMDH	bbc	bb	bbb	bbb	b	b
mMDH	bbb	bb	bbb	bbb	b	b
sMDHP	aaa	aa	aaa	aaa	b>a	b>a
sIDH	aab	ab	abb	abb	b	b
mIDH	aaa	aa	aaa	aaa	a	a
sSOD	abb	ab	aab	aab	a	a
mSOD	aaa	aa	aaa	aaa	a	a
DDH	aaa	aa	aaa	aaa	a	a
sAAT	bcc	bc	bbc	bbc	b	b
mAAT	aaa	ab	abb	abb	b	b>c
AK	aaa	aa	aaa	aaa	a	a
CK1	bbb	bb	bbb	bbb	b	b
CK2	—	aa	aaa	aaa	a	a
EST2[d]	a+a+a+	a+a	a+ab	a+ab	a,b	a,b
ESTD	bbb	bc	bbc	bbc	b	b
PEPA	aac	ac	abc	abc	b	b
PEPB[d]	bd+d+	cd	ccd	bcd	b,c	b
PEPD	ccc	dd	add	bdd	a,b,c,e	a,b,c,d,e
sACOH	bbb	bc	bbc	bbc	b	b
MPI	bbb	ab	abb	abb	b	b
GPI	bbb	bb	bbb	bbc	a,b,c	c
PGM1	—	aa	aaa	aaa	a	a
PGM2	dde	dd	ddd	ddd	d	d
PGM3	bbd	ac	aac	aac	a≫b,c	a≫b
TF	abb	ab	abb	abb	b>a	b>a
HB	aaa	aa	aaa	aaa	a	a
12S mtDNA	—	M	M	M	P>M	P

[a] Sites 2 and 10 are comprised largely of *C. t. punctilinealis* (PUN). The contact region and collecting sites are described and mapped in table 2, appendix 1, and figures 1 and 3.

[b] Ploidy.

[c] Allozymes of alleles listed correspond to those observed in *C. tigris* (tables 5–12; appendix 3). The 12S mtDNA data refer to allele-specific oligonucleotides (table 4).

[d] Allele a+ and allele d+ of *C. uniparens* and *C. neomexicanus* (EST2 and PEPB) were not observed in any *C. tigris*. These alleles had mobilities faster than the a- and d-alleles observed in *C. tigris* or *C. neomexicanus*.

the a-allele at IDDH, lack a second b-allele at LDH1 (fig. 52), lack the c-allele at sMDH, lack a second a-allele at sIDH, lack a second b-allele at sSOD, lack a second c-allele at sAAT, lack the second and third a-alleles at mAAT, lack the second and third a+-alleles at EST2, lack d+-alleles at PEPB, lack c-

alleles at PEPD, lack the e-allele at PGM2, and lack the b- and d-alleles at PGM3.

The possibility that *C. tigris* was the only species involved in producing these unusual specimens was ruled out because these two lizards possessed the following five alleles at five loci that were not found in any *C. tigris*

			Genotype LDH2	Genotype LDH1
UNI			-	bbb
NEO			-	ab
HYB			-	aab
NEO			-	ab
PUN			-	aa
MAR			-	aa
UNI			bbb	bbb
NEO			bb	ab
HYB			bbb	aab
NEO			bb	ab
PUN			bb	aa
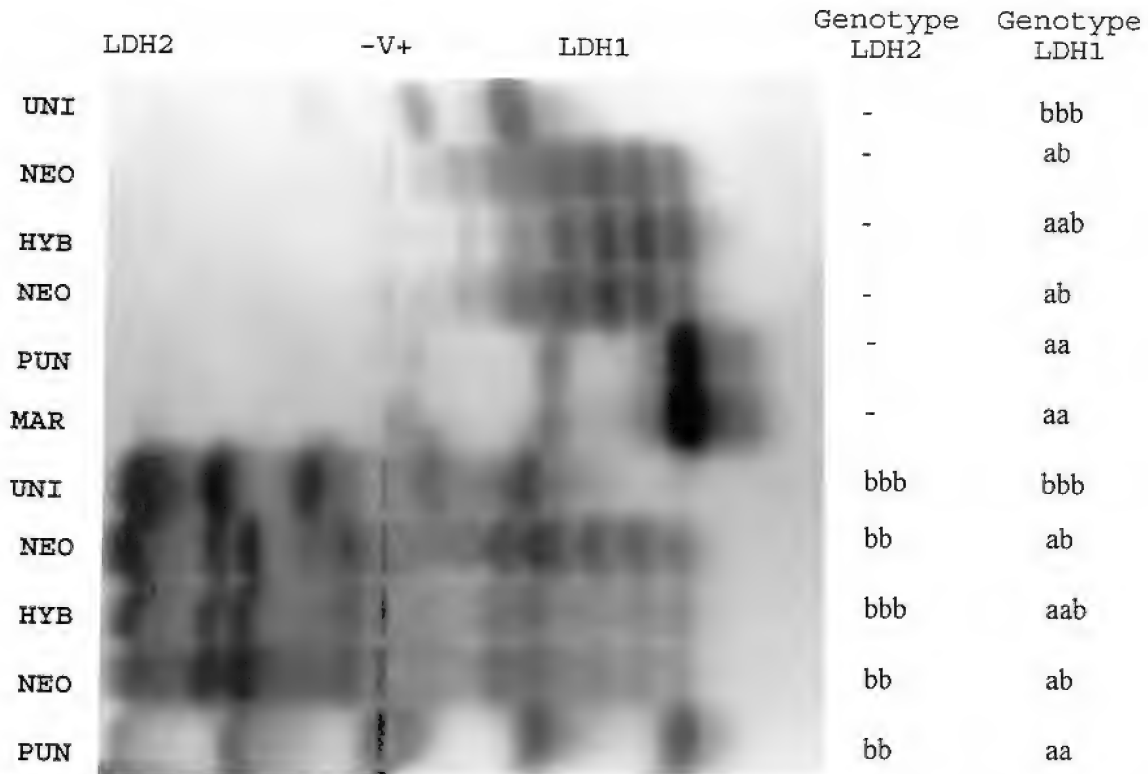
Fig. 52. Differences in the tissue distribution of lactate dehydrogenase, a tetramer. **Top** (six lanes). LDH1 predominates in heart. **Bottom** (five lanes). Both LDH1 and LDH2 are active in liver and the banding patterns include numerous isozymes composed of subunits of both. Note the five-banded patterns for LDH1 for heterozygous diploid *C. neomexicanus* (NEO) and a triploid hybrid (HYB) of *neomexicanus* × *tigris*. In the heart tissue, LDH1 genotype ab for *neomexicanus*, the isozymes approximate activities of 1:4:6:4:1. For the triploid hybrid with genotype aab, the faster migrating isozymes stain most intensely (activities approximate the theoretically expected ratio of 16:32:24:8:1). These patterns are consistent with the origin of the hybrid from a mating between *C. neomexicanus* (NEO) and *C. t. punctilinealis* (PUN). Other abbreviations are: UNI, *C. uniparens*; MAR, *C. t. marmoratus*. Arrow indicates sites of sample application; anode is to the right.

from the contact region (table 33): the c-allele at ADH, the b-allele at LDH1 (fig. 52), the b-allele at sSOD, the a+-allele at EST2, and the c-allele at ESTD. All of these alleles are found in *C. neomexicanus*, however, and indeed the genomes of these two unusual individuals are fully consistent with expectations for triploid hybrids between *C. neomexicanus* ♀ × *C. tigris* ♂ (table 33). The genotypes of these hybrids also included the two orphan alleles (at ESTD and PEPB) characteristic of *C. neomexicanus* (Cole et al., 1988).

Considering the known ancestry of *C. neomexicanus* (reviewed in Cole et al., 1988), the three haploid genomes of these triploid hybrids came together as follows: (*C. t. marmoratus* ♀ × *C. inornatus* ♂) × *C. tigris* ♂ of the contact region. The genotypes of the two hybrid lizards are very similar to each other but not identical. Their allele combinations differed by one allele each at PEPB, PEPD, and GPI (table 33). These three differences are at loci that are polymorphic with the same alternative alleles present in the *C. tigris* of the contact region. For each hybrid (from sites 2 and 10) the paternal *C. tigris* was from a site where there are alleles of both *C. t. punctilinealis* and *C. t. marmoratus*, with a preponderance of *punctilinealis* alleles. Site 2 was a hybrid locality, while lizards from site 10 primarily
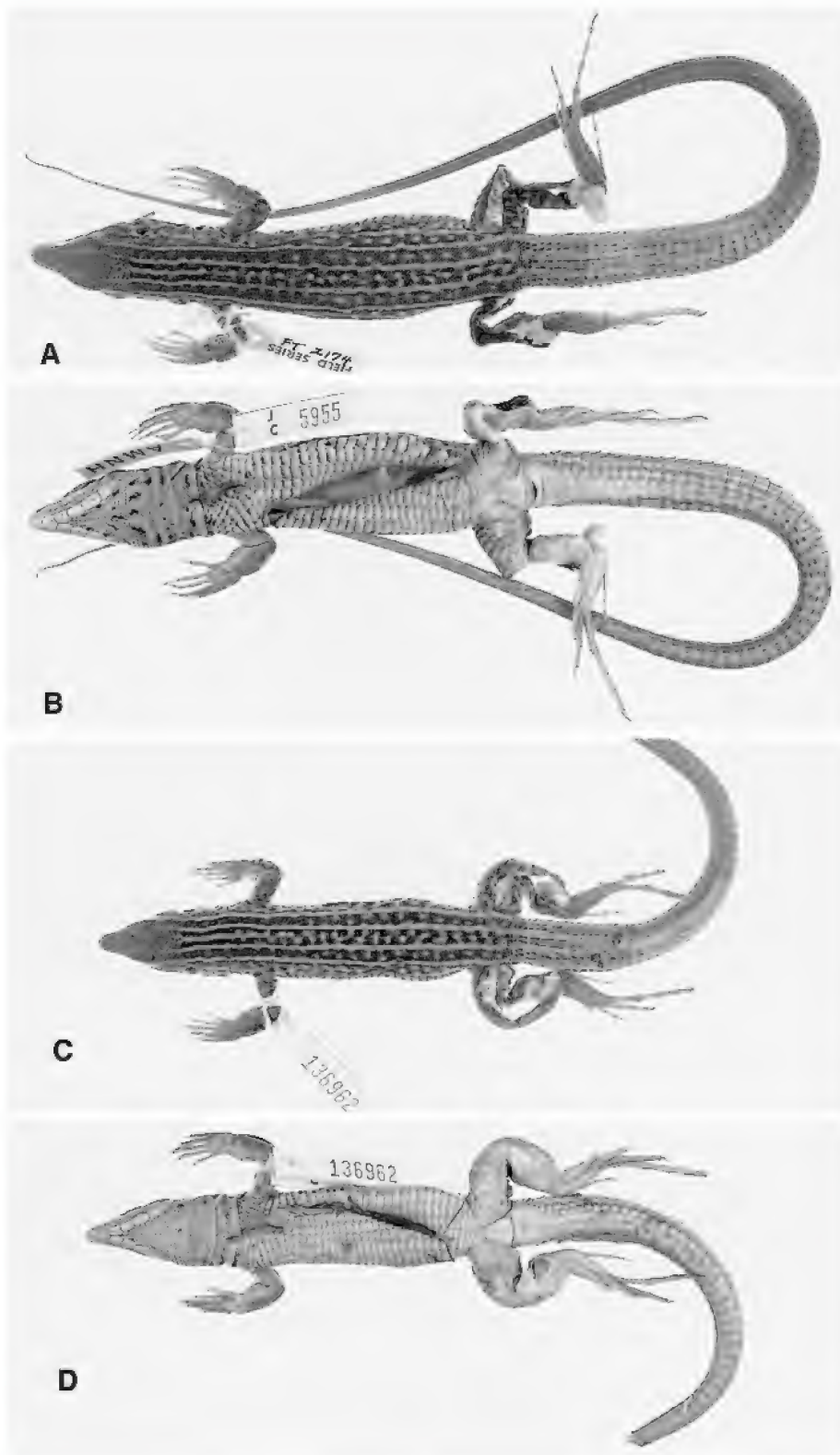
TABLE 34
Size and Scalation Data for Two Species of *Cnemidophorus* and Two of Their Interspecific Hybrids

Character	*tigris* 2[a]	*tigris* 10[b]	Hybrid 2[c]	Hybrid 10[d]	neomexi-canus[e]
S-V (mm)[f]	89.8±1.43 (84–96)9	84.7±2.96 (71–100)10	85	96	69.2±0.98 (62–78)21
Circumorbs.[g]	13.4±0.67 (11–17)9	14.9±0.61 (13–18)9	17	21	23.8±0.36 (21–27)20
Gulars[h]	23.7 (21–26)6	25.0±0.76 (21–29)9	19	22	18.5±0.70 (15–21)11
SAB[i]	93.3±2.03 (85–101)9	92.3±2.52 (86–110)9	80	79	81.2±0.65 (77–87)21
Fem. pore[j]	38.4±0.76 (35–42)9	37.0±0.99 (33–42)10	41	42	40.2±0.34 (37–43)21
Toe[k]	65.1±1.06 (59–71)9	62.7±0.90 (58–66)9	33[l]	68	66.5±0.45 (63–70)16
Mesop.[m]	No	No	Yes	No	Yes

[a] Based on specimens from site 2.

[b] Based on specimens from site 10.

[c] Hybrid from site 2, AMNH R-136962 (fig. 53).

[d] Hybrid from site 10, AMNH R-137669 (fig. 53).

[e] Data from Cole et al. (1988: 18; their table 2, Lordsburg column).

[f] Snout–vent length. Data presented are the mean ± one standard error of the mean (range) *N*.

[g] Total number of circumorbital scales.

[h] Total number of gulars, including the anteriormost medial gular scale if present (for consistency with data for *neomexicanus*).

[i] Scales around midbody.

[j] Total number of femoral pores.

[k] Total number of fourth toe lamellae.

[l] The right fourth toe is missing for this hybrid, so only the left toe could be counted. The total for both toes probably was 66.

[m] "No" means that the mesoptychial scales are not abruptly enlarged; "yes" means that they are somewhat so, typical for *neomexicanus*.

appeared morphologically to be *punctilineal-is*, although they contained 20% of the diagnostic alleles of *marmoratus*.

The specimens of *C. neomexicanus* × *C. tigris* are illustrated in figure 53. Both individuals had scores of 0.17 on the morphological hybrid index for *C. tigris*, as both were similar to *punctilinealis* in four characters, but they appeared to be F₁ hybrids in chin and chest coloration. On retrospective examination, however, the *neomexicanus*-like wavy vertebral light stripe on the neck of both specimens (although not continuous along the body) reflected the typical condition of their maternal parent.

In characters of size and scalation (table 34), the hybrids showed intermediacy between their two parental forms or were more similar to one parent than the other. In body length, both hybrids resembled *C. tigris* rather than *C. neomexicanus*, but in number of scales around midbody, both resembled *neomexicanus* rather than *tigris*. In number of circumorbital scales and gulars, and in rela-

←

Fig. 53.　Two hybrids of *C. neomexicanus* × *C. tigris*. **A**. Dorsal view, AMNH R-137669 from site 10, body length 96 mm. **B**. Ventral view of AMNH R-137669. **C**. Dorsal view, AMNH R-136962 from site 2, body length 85 mm. **D**. Ventral view of AMNH R-136962.

tive size of the mesoptychials, one hybrid resembled *neomexicanus* while the other resembled *tigris*.

As discussed for other hybrids between unisexual and bisexual taxa (for example, Hardy and Cole, 1998), hybrids should strongly resemble the maternal parent, as they inherit 100% of her genome in the cloned egg and only a haploid complement from the male. At first glance for these triploids, one might suggest that the hybrids would have received only 33% of their genes from *C. tigris*. However, a much greater resemblance to *tigris* is expected for these individuals because of the ancestry of the maternal parent, *C. neomexicanus*: *C. t. marmoratus* ♀ × *C. inornatus* ♂. Consequently, these hybrids received two of their three haploid genomes ultimately from *C. tigris*, involving two subspecies, which explains their similarity to the *tigris* of the contact region.

Both hybrids were females of mature body size (table 34), but they appeared to be sterile. Their ovaries and visible oocytes are small, and the oviducts are narrow (not expanded), as if no ova have passed through them.

Although we did not karyotype these individuals, the fact that they are females suggests that they inherited the X chromosome of *C. tigris* in the most recent fertilization event (Cole et al., 1969; Bull, 1978; Cole, 1979).

HYBRIDIZATION WITH THE UNISEXUAL *C. TESSELATUS*

In the course of conducting extensive ecological research with *Cnemidophorus dixoni* (= *C. tesselatus* type F; Zweifel, 1965), for which thousands of specimens of *Cnemidophorus* were handled in Antelope Pass, west of Animas, Hidalgo County, New Mexico, Charles W. Painter and colleagues found a few hybrids of *C. tesselatus* × *C. t. punctilinealis* (Painter, personal commun.). Our collaborative research with Painter involving the genetics and origins of these animals will be presented in a separate report. In addition, Painter and collaborators found three specimens of *C. tigris* in the vicinity of the eastern end of Antelope Pass bearing a few traits of *C. t. marmoratus*, suggesting that limited gene flow between *punctilinealis* and *marmoratus* occurs there now. We suspect that the *marmoratus* genes moved southward along the eastern slopes of the Peloncillo Mountains from the vicinity of the southern hybrid zone.

Cnemidophorus t. marmoratus also hybridizes frequently with *C. tesselatus* type E in the vicinity of Roswell, Chaves County, New Mexico (H. L. Taylor, et al., in prep.). To date, the numerous hybrids found at that locality appear to be triploid, F_1 individuals, with a 50:50 sex ratio. Our collaborative research with Taylor involving the genetics and origins of these animals will also be presented in a separate report.

SUMMARY AND CONCLUSIONS

1. *Cnemidophorus tigris punctilinealis* inhabits desertscrub in the northeastern Sonoran Desert.

2. *Cnemidophorus tigris marmoratus* inhabits desertscrub in the northwestern Chihuahuan Desert, and it differs from *punctilinealis* in six characters of coloration.

3. These two taxa, which share a common ancestor, today converge in desert and semidesert habitats and interbreed in three or more secondary contact zones in the northwestern Animas Valley of southwestern New Mexico.

4. Genetic and morphological studies were conducted on 607 lizards from 48 sites within the approximately 60 × 75 km contact region in SW New Mexico and adjacent SE Arizona.

5. Transects across each of three hybrid zones revealed significant shifts in allele frequencies at five protein loci (IDDH, sMDHP, EST2, PEPB, and TF), in alternative haplotypes of mitochondrial DNA, in the six characters of coloration, and in a few poorly differentiated characters of scalation and additional proteins (for example, PEPD and GPI).

6. The frequency shifts for all characters form sharp step-clines that are concordant and coincident in each hybrid zone.

7. In addition, the clines of all three tran-

sects of the separate hybrid zones are similar to each other in width and shape.

8. The hybrid zones are approximately 3–8 km wide (based on the frequencies of *marmoratus* alleles being from 0.2–0.8), although genetic introgression extends far beyond this distance.

9. Hardy-Weinberg, nuclear linkage, and cytonuclear equilibria prevail, and there is no clear genetic, morphological, reproductive, or physiological evidence for selection against hybrids. Random, nonassortative mating (panmixia) prevails in each hybrid zone.

10. Estimates of gene flow indicate that the clines resulted from neutral secondary contact initiated with the newest reconnection of the Sonoran and Chihuahuan Deserts within the present interglacial episode, from 1000 to 5000 years ago, consistent with paleoecological data from packrat middens.

11. Certain hypotheses concerning the origin of uncommon alleles in hybrid zones and asymmetry in hybrids are not supported by our analyses of these lizards. Uncommon alleles (frequency < 0.02) were not associated with the centers of the hybrid zones. Their discovery appeared to be largely a function of sample size.

12. Specimens from the hybrid zones showed no signs of greater asymmetry in scalation than did nonhybrids.

13. No clear evidence was found to support our earlier suggestion that the central hybrid zone may be moving eastward.

14. We present detailed habitat notes, vegetation maps, site descriptions with GPS-determined coordinates, dated photographs, and gene frequency data in order to provide a baseline against which future comparisons can be made.

15. The patterns of present-day habitat distributions and lizard gene frequencies at the hybrid and nonhybrid sites probably reflect Pleistocene–Recent expansion of the Sonoran Desert eastward and the Chihuahuan Desert westward during the present interglacial period in which we live, with natural climatic changes perhaps having human-mediated effects superimposed on them in the most recent 130 years.

16. Contact and separation of these subspecies of *C. tigris* probably occurred repeatedly during interglacial and glacial episodes due to climatic changes, even though the nearest glaciers were not within hundreds of miles of the contact region.

17. Sex ratios in the contact region are consistent with Mendelian patterns of inheritance and the allele frequencies observed among these lizards, suggesting that no unisexual clones of hybrid origin are emerging among the *C. t. punctilinealis* × *C. t. marmoratus* hybrids at this time.

18. A glance at the steep step-clines in the hybrid zones can be misleading, as the genetic differences among the subspecies appear to be smaller when viewed in the context of additional samples taken distant from the contact region and comparisons with the nearby subspecies *C. t. aethiops* and *C. t. septentrionalis*. If subspecies of *Cnemidophorus tigris* are to be recognized at all, it is reasonable and consistent in practice to include *marmoratus* among them.

19. On occasions, *Cnemidophorus tigris* also hybridizes with the diploid and unisexual *C. neomexicanus* in the contact region and *C. tesselatus* type F (= *C. dixoni*) nearby to the south. These events do not appear to be producing new unisexual triploid clones at this time, as the hybrids examined to date appear to be sterile. Investigators who initiate research with these organisms in the future should be aware that these rare hybrids can be easily misidentified by morphological criteria.

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APPENDIX 1

Specimens Examined from the Contact Region

The specimens are listed in numerical order of the collecting sites (fig. 3, table 2), and full locality data and habitat descriptions are presented as noted in the field (distances originally noted in miles were multiplied by 1.61 for conversion to kilometers; elevations in feet were multiplied by 0.3048 for conversion to meters). AMNH refers to individual catalog numbers at the American Museum of Natural History. FT (frozen tissue) and HCD (Dessauer) refer to frozen tissue catalog numbers of the AMNH or Louisiana State University (LSU). The AMNH, FT, and HCD specimen numbers are not necessarily presented in sequence, so readers who need those numbers correlated on an individual specimen basis should contact the AMNH, where the numbers are correlated in the catalogs. "Sample" refers to population sample numbers that were assigned to collections as they were received, stored, and analyzed at LSU. These sample numbers are not to be confused with site numbers. The site numbers were assigned for efficient communication in this volume. The cross references are provided here in case future researchers need to correlate information from laboratory records (sample and FT numbers) and this volume (site numbers).

Dates of collection are between 28 July 1981 and 10 July 1994. The specific date for each specimen is available from the AMNH. The sequence of plants listed for each site indicates relative areas covered at the site from most to least abundant. Common names for the plants mentioned most frequently are: creosote bush (*Larrea divaricata*), mesquite (*Prosopis*), tarbush (*Flourensia cernua*), snakeweed (*Gutierrezia*), catclaw (*Acacia greggii*), crucifixion thorn (*Koeberlinea*),

Mormon tea (*Ephedra*), yucca (*Yucca*), one-seed juniper (*Juniperus monosperma*), and desert willow (*Chilopsis linearis*).

Virtually all of the following 612 specimens were used for examining coloration and pattern, the full suite of 36 gene loci analyzed electrophoretically, mtDNA, sex ratio, and reproductive state. A few additional specimens, used principally for biochemical work, were not saved as vouchers. The subsamples used to analyze size, scutellation, and karyotypes are identified in the relevant sections of the text.

SITE 1: New Mexico: Grant County; 34.9 km (by NM Hwys 70 and 464) N of Lordsburg [Hidalgo County]; 1280 m elev; collected on both sides of the road. AMNH R-136966–75; FT 2076–81 and 2092–95; sample 34. Habitat: sandy soil, riparian canyon bottom, tributary of the Gila River; most abundant bushes are *Acacia greggii* and *Prosopis*; *Juniperus monosperma*, *Yucca elata*, *Gutierrezia*, and other shrubs and grasses were present, but no cacti were seen. Specimens are *C. t. punctilinealis*.

SITE 2: New Mexico: Grant County; 30.1 km (by NM Hwys 70 and 464) N of Lordsburg [Hidalgo County]; 1370 m elev; collected on both sides of the road. AMNH R-136956–65; FT 2026 and 2048–56; sample 35. Habitat: sandy wash with numerous *Chilopsis linearis* in *Prosopis–Yucca* grassland; *Gutierrezia*, *Ephedra* and other small shrubs, and prickly pear cactus (*Opuntia*), but few cholla and no *Larrea* were seen. Specimens look largely like *C. t. punctilinealis* but genetically are hybrids with *C. t. marmoratus*, excepting AMNH R-136962, which is a *C. neomexicanus* × *C. tigris* hybrid.

SITE 3: New Mexico: Hidalgo County; 28.6 km (by NM Hwys 70 and 464) N of Lordsburg; 1370 m elev; collected on both sides of the road. AMNH R-136946–55 and 139714–23; FT 2027–30, 2057–62, 6724–27, and 6736–41; sample 36. Habitat: sandy wash similar to site 2 but with fewer *Chilopsis* and here there are some (very few, scattered) *Larrea* up out of the wash on the N side. Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids.

SITE 4: New Mexico: Hidalgo County; 27.0 km (by NM Hwys 70 and 464) N of Lordsburg; 1400 m elev; collected on the W side of the road. AMNH R-136936–45; FT 2031–34 and 2063–68; sample 37. Habitat: open *Prosopis–Yucca* grassland on sandy soil with *Gutierrezia*, other small shrubs, *Ephedra*, and cholla and prickly pear cactus (*Opuntia*). Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids.

SITE 5: New Mexico: Hidalgo County; 25.4 km (by NM Hwys 70 and 464) N of Lordsburg; 1400 m elev; collected on the W side of the road. AMNH R-136926–35; FT 5823–25 and 5857–63; sample 38. Habitat: similar to site 4, but no *Ephedra* seen. Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids.

SITE 6: New Mexico: Hidalgo County; 23.8 km (by NM Hwys 70 and 464) N of Lordsburg; 1400 m elev; collected on the W side of the road. AMNH R-136916–25; FT 2038–47; sample 39. Habitat: *Prosopis–Yucca* grassland on sandy soil with *Gutierrezia* and other small shrubs, and cholla and prickly pear cacti (*Opuntia*), but little *Ephedra*. Specimens are largely *C. t. marmoratus*, but with *punctilinealis* influence.

SITE 7: New Mexico: Hidalgo County; 18.4 km (by NM Hwys 70 and 464) N of Lordsburg; 1400 m elev; collected on both sides of the road. AMNH R-136906–15; FT 2082–91; sample 40. Habitat: wash in *Prosopis–Yucca* grassland on sandy soil with *Ephedra*, *Gutierrezia*, other small shrubs, and cholla and prickly pear cacti (*Opuntia*). Specimens are *C. t. marmoratus*.

SITE 8: New Mexico: Hidalgo County; 28.5 km (by NM Hwys 70 and 464) N of Lordsburg, then 6.0 km (by gravel road) W; 1340 m elev; collected on the N side of the road. AMNH R-137676 and 138552; FT 2210 and 6325; sample 53. Habitat: *Larrea* desertscrub. One specimen looks like *C. t. punctilinealis*, the other like *marmoratus*.

SITE 9: New Mexico: Hidalgo County; 31.7 km (by NM Hwy 70) NW of Lordsburg, then 6.1 km (by gravel road) ENE; 1310 m elev; collected on the N side of the road. AMNH R-138557–66; FT 6330–39; sample 58. Habitat: *Larrea* desertscrub on sandy soil with grass, *Koeberlinea*, *Gutierrezia*, cholla and prickly pear (*Opuntia*) cacti,

Yucca, and very few widely scattered *Prosopis*. Specimens are largely *C. t. punctilinealis*, but with considerable *marmoratus* influence.

SITE 10: New Mexico: Hidalgo County; 31.7 km (by NM Hwy 70) NW of Lordsburg; 1280 m elev; collected on the N side of Hwy 70, both sides of the gravel road. AMNH R-136893–95, 137665–71, and 138567; FT 2136, 2141–42, 2170–76, and 6340; sample 44. Habitat: *Larrea* desertscrub with grass. Specimens listed are *C. t. punctilinealis*, excepting AMNH R-137669, which is a *C. neomexicanus* × *C. t. punctilinealis* hybrid.

SITE 11: New Mexico; Hidalgo County; 34.9 km (by NM Hwy 70) NW of Lordsburg; 1280 m elev; collected on the S side of Hwy 70. AMNH R-138538–46 and 138553; FT 6311–19 and 6326; sample 57. Habitat: *Larrea* desertscrub on sandy soil with gravel, abundant *Prosopis* also, *Gutierrezia*, *Koeberlinea*, cholla and prickly pear (*Opuntia*) cacti, and *Yucca*. Specimens are *C. t. punctilinealis*.

SITE 12: New Mexico: Hidalgo County; 26.7 km (by NM Hwy 70) NW of Lordsburg, then 10.3 km (by gravel road) SW (site is 1.1 km E of Summit); 1310 m elev; collected on the S side of the gravel road. AMNH R-138580–82 and 138584–90; FT 6353–55, 6357, and 6359–64; sample 70. Habitat: *Larrea* desertscrub on sandy soil with gravel, *Flourensia*, *Gutierrezia*, grass, some *Yucca*, and a few *Ephedra* and prickly pear cacti (*Opuntia*). Most of the specimens are clearly *C. t. punctilinealis*, but one is referable to *marmoratus*.

SITE 13: New Mexico: Hidalgo County; 23.2 km (by NM Hwy 70) NW of Lordsburg; 1310 m elev; collected on the N side of Hwy 70. AMNH R-138568–73 and 138575–78; FT 6341–46 and 6348–51; sample 59. Habitat: *Prosopis–Yucca* grassland on loose sand with abundant grass between shrubs, *Gutierrezia*, a few cholla and prickly pear (*Opuntia*) cacti, very few *Ephedra*, and one *Acacia greggii* seen. Specimens are *C. t. marmoratus*.

SITE 14: New Mexico: Hidalgo County; 16.3 km (by NM Hwy 70) NW of Lordsburg; 1310 m elev; collected on the S side of Hwy 70. AMNH R-120693–95, 127004–06, 127101–111, 131082–88, 131090, 131092–99, and 133084–128; FT 282–84, 343, 345–48, 516–30, 544–47, 581, 588–89, 892–95, 1108–09, 1111, 1121–22, 1233, 1501, 1718–21, 1723–28, 1730, 1794, 1796–98, 1801–12, 1818–24, 1840–49, 1860; and HCD 5603–04; 10 of the listed specimens were frozen whole, without preserved vouchers (no AMNH numbers); sample 9. Habitat: loose sand in hummocks with *Prosopis* and little grass between bushes. Specimens are *C. t. marmoratus*.

SITE 15: New Mexico: Hidalgo County; 11.3

km (by NM Hwy 70) NW of Lordsburg; 1310 m elev; collected on the N side of Hwy 70. AMNH R-120691, 122831, and 131102; FT 281, 329, and 344; sample 10. Habitat: *Prosopis*–grassland. Specimens are *C. t. marmoratus*.

SITE 16: New Mexico: Hidalgo County; 17.2 km (by gravel road) N of Steins; 1310 m elev; collected on both sides of the gravel road. AMNH R-138511–16 and 138547–50; FT 6255–56, 6258–61, and 6320–23; sample 55. Habitat: *Larrea* desertscrub with *Flourensia*, *Gutierrezia*, grass, *Yucca*, cholla and prickly pear (*Opuntia*) cacti, *Koeberlinea*, and, in the wash, desert sumac (*Rhus microphylla*) and thistles. Specimens are *C. t. marmoratus*.

SITE 17: New Mexico: Hidalgo County; 10.6 km (by gravel road) N of Steins; 1310 m elev; collected on both sides of the gravel road. AMNH R-136902; FT 2152; sample 43. Habitat: *Larrea* desertscrub with gravel, grass, *Yucca*, *Ephedra*, *Acacia greggii*, *Gutierrezia*, *Flourensia*, and two barrel cacti seen. The specimen is *C. t. marmoratus.*

SITE 18: New Mexico: Hidalgo County; 9.5 km (by gravel road) N of Steins; 1310 m elev; collected on both sides of the gravel road. AMNH R-136903–05, 137687–90, 138551, and 138554–55; FT 2153–54, 2165, 2177–80, 6324, and 6327–28; sample 42. Habitat: *Larrea* desertscrub on sandy soil with gravel, *Flourensia*, grass, widely scattered cholla and prickly pear (*Opuntia*) cacti, *Koeberlinea*, *Gutierrezia*, and widely scattered *Yucca*. Site is 0.7 km S of a pipeline road. Specimens are largely *C. t. marmoratus*, but with *punctilinealis* influence.

SITE 19: New Mexico: Hidalgo County; 4.8 km (by gravel road) N of Steins; 1310 m elev; collected on both sides of the gravel road. AMNH R-137677–86; FT 2184–93; sample 54. Habitat: *Larrea* desertscrub on soil with gravel, some *Flourensia*, grass, *Gutierrezia*, *Yucca*, a few *Prosopis*, a few *Acacia greggii*, and a few cholla. Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids.

SITE 20: Arizona: Cochise County; 6.4 km (linear) NE of San Simon; 1160 m elev; collected on the W side of the gravel road. AMNH R-137655–64, 138497–508, and 139694–703; FT 2194–203, 6283–94, and 6713–22; sample 52. Habitat: *Larrea* desertscrub on soil with gravel, some *Acacia greggii*, considerable *Gutierrezia*, few cholla and prickly pear (*Opuntia*) cacti, few *Yucca*, and very few *Prosopis* and barrel cacti. Specimens are *C. t. punctilinealis*.

SITE 21: Arizona: Cochise County; Cavot Road, 0.5 km SW of Hwy I-10 (site is 6.9 km W of Steins, Hidalgo Co., NM); 1220 m elev.; collected on the S side of the gravel road. AMNH

R-138486 and 138488–96; FT 6262 and 6264–6272; sample 56. Habitat: *Larrea* desertscrub on sand with gravel, *Flourensia*, *Gutierrezia*, a few cholla, barrel, and prickly pear (*Opuntia*) cacti, few *Yucca*, few ocotillo (*Fouquieria splendens*); desert sumac (*Rhus microphylla*) and *Acacia greggii* abound in the wash. Specimens are *C. t. punctilinealis*.

SITE 22: New Mexico: Hidalgo County; 1.0 km (by NM Hwy 80) S of Road Forks, then 5.0 km (by pipeline road) W; 1325 m elev; collected on both sides of pipeline road. AMNH R-128357–66; FT 1019–28; sample 29. Habitat: *Larrea* desertscrub on soil with gravel, abundant *Acacia greggii*, abundant *Gutierrezia*, with *Flourensia*, cholla, and widely scattered *Yucca*, prickly pear cacti (*Opuntia*), and *Juniperus monosperma*. Specimens are *C. t. punctilinealis*. This was published as sample 2 in Dessauer and Cole (1991).

SITE 23: New Mexico; Hidalgo County; 0.5 km (linear) S and 0.3 km (linear) E of Steins; 1340 m elev; collected on both sides of pipeline road. AMNH R-128373–82; FT 968–77; sample 24. Habitat: *Larrea* desertscrub on sand with gravel, *Flourensia*, *Acacia greggii*, *Gutierrezia*, *Koeberlinea*, *Ephedra*, *Yucca*, *Prosopis*, and cholla and prickly pear (*Opuntia*) cacti. Specimens are largely *C. t. punctilinealis*, but with *marmoratus* influence. This was published as sample 3 in Dessauer and Cole (1991).

SITE 24: New Mexico: Hidalgo County; 1.0 km (by NM Hwy 80) S of Road Forks, then 2.4 km (by pipeline road) W; 1310 m elev; collected on both sides of pipeline road. AMNH R-128403–12; FT 1009–18; sample 28. Habitat: *Larrea* desertscrub on sandy soil with gravel, *Gutierrezia*, and very few each of *Flourensia*, *Acacia greggii*, *Yucca*, *Prosopis*, *Koeberlinea*, and prickly pear (*Opuntia*) cacti. Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids. This was published as sample 4 in Dessauer and Cole (1991).

SITE 25: New Mexico: Hidalgo County; 1.0 km (by NM Hwy 80) S of Road Forks, then 1.6 km (by pipeline road) W; 1310 m elev; collected on both sides of pipeline road. AMNH R-128383–92; FT 978–87; sample 25. Habitat: *Larrea* desertscrub on sandy soil with gravel, *Flourensia*, *Gutierrezia*, *Koeberlinea*, and prickly pear cactus (*Opuntia*). Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids. This was published as sample 5 in Dessauer and Cole (1991).

SITE 26: New Mexico: Hidalgo County; 1.0 km (by NM Hwy 80) S of Road Forks, then 1.1 km (by pipeline road) W; 1310 m elev; collected on both sides of pipeline road. AMNH R-131420–29, 138510, 138517–26, and 139724–33; FT 1617–24, 1631–32, 6252, 6273–82, 6728–35, and 6743–44; sample 32. Habitat: *Larrea* desertscrub.

Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids. This was published as sample 6 in Dessauer and Cole (1991).

SITE 27: New Mexico: Hidalgo County; 1.0 km (by NM Hwy 80) S of Road Forks, then 0.8 km (by pipeline road) W; 1310 m elev; collected on both sides of pipeline road. AMNH R-128393–402; FT 998–1007; sample 27. Habitat: *Larrea* desertscrub on sand with gravel, *Koeberlinea*, prickly pear cactus (*Opuntia*), and very little *Flourensia*. Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids. This was published as sample 7 in Dessauer and Cole (1991).

SITE 28: New Mexico: Hidalgo County; 1.0 km (by NM Hwy 80) S of Road Forks, at pipeline road junction; 1280 m elev; collected on both sides of pipeline road. AMNH R-128335–44; FT 988–997, 997b, and 997c (the last two specimens were frozen whole and have no vouchers); sample 26. Habitat: *Larrea* desertscrub on sand with gravel, *Flourensia*, *Gutierrezia*, *Koeberlinea*, prickly pear cactus (*Opuntia*), and *Ephedra*. Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids. This was published as sample 8 in Dessauer and Cole (1991).

SITE 29: New Mexico: Hidalgo County; 11.3 km WSW of Lordsburg (site is 0.6 km S of Hwy I-10); 1280 m elev. AMNH R-136896–901, 137672–75, 138527–36, and 139704–13; FT 2159–64, 2166–69, 6295–305, and 6745–54; sample 41. Habitat: *Larrea* desertscrub on sand with gravel, *Prosopis*, *Flourensia*, fourwing saltbush (*Atriplex canescens*), *Gutierrezia*, and other small shrubs. Specimens are *C. t. marmoratus*.

SITE 30: New Mexico: Hidalgo County; 2.7 km (by NM Hwy 80) S of Road Forks, then 0.8 km (by gravel road) E; 1310 m elev; collected on both sides of the gravel road. AMNH R-128345–54; FT 1032–41; sample 30. Habitat: primarily *Prosopis* on sandy soil with *Flourensia*, *Koeberlinea*, and *Gutierrezia*. Specimens are largely *C. t. marmoratus*, but with *punctilinealis* influence. This was published as sample 9 in Dessauer and Cole (1991).

SITE 31: New Mexico: Hidalgo County; 6.3 km (by NM Hwy 80) S of Road Forks, then 0.6 km (by gravel road) E; 1310 m elev; collected on both sides of the gravel road. AMNH R-127078–87; FT 768–77; sample 13. Habitat: *Larrea* desertscrub on loose sandy soil, with *Gutierrezia*, *Flourensia*, other shrubs, and widely scattered *Yucca*; in the wash are large, dense *Acacia greggii* and *Koeberlinea*. Specimens are *C. t. marmoratus*.

SITE 32: New Mexico: Hidalgo County; 9.3 km (by NM Hwy 80) S of Road Forks, then 0.3 km (by gravel road) E; 1310 m elev; collected on both sides of the gravel road. AMNH R-127190–

99; FT 778–87; sample 22. Habitat: *Larrea* desertscrub on soil with gravel, *Gutierrezia*, *Koeberlinea*, *Flourensia*, other shrubs, and very few scattered *Yucca*. Specimens are largely *C. t. marmoratus*, but with *punctilinealis* influence.

SITE 33: New Mexico: Hidalgo County; 12.4 km (by NM Hwy 80) S of Road Forks, then 0.8 km (by paved road) E; 1280 m elev; collected on both sides of the small paved road. AMNH R-127140–49; FT 698–707; sample 21. Habitat: *Larrea* desertscrub on soil with gravel, *Gutierrezia*, some desert sumac (*Rhus microphylla*), very few *Prosopis*, *Acacia greggii*, whitethorn (*Acacia constricta*), *Koeberlinea*, prickly pear cactus (*Opuntia*), and very few cholla. Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids.

SITE 34: New Mexico: Hidalgo County; 20.4 km (linear) S and 1.6 km (linear) E of Road Forks; 1300 m elev; collected on both sides of the gravel road. AMNH R-127180–89; FT 748–57; sample 14. Habitat: *Larrea* desertscrub on soil with gravel, *Gutierrezia*, *Flourensia*, *Koeberlinea*, other shrubs, and a few prickly pear cacti (*Opuntia*). We collected on the N side of the road. Specimens are *C. t. punctilinealis*. We reached this site by driving 15.4 km S of Road Forks on NM Hwy 80, then 6.3 km to the SSE on a gravel road, to the eastern end of a sharp bend to the east.

SITE 35: New Mexico: Hidalgo County; 23.3 km (linear) S and 3.2 km (linear) E of Road Forks; 1310 m elev. AMNH R-127027–36; FT 788–97; sample 6. Habitat: *Prosopis* semidesert grassland on sandy soil with graythorn (*Condalia lycioides*), *Koeberlinea*, *Flourensia*, *Gutierrezia*, and Russian thistle (*Salsola*). Specimens are *C. t. punctilinealis*.

SITE 36: Arizona: Cochise County; 4.8 km (linear) E and 16.1 km (linear) S of San Simon; 1220 m elev; collected 0.2 km W of Portal to San Simon (gravel) Road. AMNH R-127047–55 and 138509; FT 679–87 and 6365; sample 2. Habitat: mixed *Larrea* and *Prosopis* desertscrub on sandy soil with some *Flourensia*, *Gutierrezia*, and a few cholla. Specimens are *C. t. punctilinealis*. This was published as sample 1 in Dessauer and Cole (1991), but here we have added a tenth specimen.

SITE 37: Arizona: Cochise County; 17.7 km (linear) S and 12.1 km (linear) E of San Simon; 1190 m elev. AMNH R-127037–46; FT 798–807; sample 3. Habitat: mixed *Larrea* and *Prosopis* desertscrub on sandy soil with *Acacia greggii*, *Flourensia*, *Koeberlinea*, scattered *Gutierrezia*, very few cholla and prickly pear (*Opuntia*) cacti, and fewer *Yucca*. Specimens are *C. t. punctilinealis*. We reached this site from Portal, AZ, as follows: go N on Foothills Road (toward San Simon) from Portal Road; after 30.3 km, turn E onto gravel road; after 4.8 km, turn S onto inconspic-

uous dirt road; after another 4.8 km, turn E at windmill; after 1.1 km road bends toward S, but continue on it; after another 1.0 km, road bends to the ESE at a gate; go 0.8 km farther and collect.

SITE 38: New Mexico: Hidalgo County; 13.7 km (linear) S and 5.6 km (linear) W of Road Forks; 1280 m elev. AMNH R-127007–16; FT 728–37; sample 4. Habitat: *Larrea* desertscrub on soil with gravel, ocotillo (*Fouquieria splendens*), and very few each of *Flourensia*, *Acacia greggii*, and *Yucca*; two *Juniperus monosperma* were within 100 m of the truck. Specimens are *C. t. punctilinealis*. We reached this site by turning NW on a gravel road at 1.9 km NE of the bend in Hwy 80 just S of the San Simon River bridge; after driving NW more or less along the river for 6.9 km (ca. 0.6 km past the track into the spillway), we turned NE on a narrow track and drove another 4.2 km.

SITE 39: New Mexico: Hidalgo County; 18.8 km (by NM Hwy 80) S of Road Forks, then 0.3 km (by gravel road) W; 1325 m elev; collected on both sides of gravel road. AMNH R-127017–26; FT 758–67; sample 5. Habitat: *Larrea* desertscrub on soil with gravel, ocotillo (*Fouquieria splendens*), *Flourensia*, *Yucca*, and cholla and prickly pear (*Opuntia*) cacti; the nearby wash has *Prosopis*, and a lone *Juniperus monosperma* was just below us on the bajada. Specimens are *C. t. punctilinealis*.

SITE 40: New Mexico: Hidalgo County; 15.4 km (by NM Hwy 80) S of Road Forks, then 0.6 km (by gravel road) NW; 1370 m elev; collected on both sides of gravel road. AMNH R-127160–69; FT 718–27; sample 16. Habitat: *Larrea* desertscrub with *Flourensia*, *Gutierrezia*, *Prosopis*, *Yucca*, and cholla and prickly pear (*Opuntia*) cacti; an adjacent wash has *Acacia greggii*, whitethorn (*Acacia constricta*), and a few widely scattered *Juniperus monosperma*. Specimens are largely *C. t. punctilinealis* but with *marmoratus* influence.

SITE 41: New Mexico: Hidalgo County; vicinity of Crystal Mine (spelled Crystle Mine on one topographic map), 13.2 km (by NM Hwy 80) S of Road Forks, then 0.3 km (by gravel road) W; 1340 m elev; collected on W side of Hwy 80, between it and the mine. AMNH R-127210–13 and 128367–72; FT 854–57, 928, 965–67, and 1045–46; sample 17. Habitat: *Larrea* desertscrub with *Flourensia*, *Gutierrezia*, whitethorn (*Acacia constricta*), and very few prickly pear cacti (*Opuntia*). Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids.

SITE 42: New Mexico: Hidalgo County; 15.4 km (by NM Hwy 80) S of Road Forks, then 1.4 km (by gravel road) SE; 1340 m elev; collected on the E side of the gravel road. AMNH R-127130–39; FT 668–77; sample 18. Habitat: *Larrea* desertscrub on sandy soil with gravel, *Gutierrezia*, scattered cholla, and a few *Yucca*. Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids.

SITE 43: New Mexico: Hidalgo County; 15.4 km (by NM Hwy 80) S of Road Forks, then 4.0 km (by gravel road) SSE; 1310 m elev; collected on the E side of the gravel road. AMNH R-127150–59; FT 708–17; sample 15. Habitat: *Larrea* desertscrub on sandy soil with *Flourensia*, *Gutierrezia*, *Yucca*, and *Koeberlinea*. Specimens are largely *C. t. punctilinealis* but with *marmoratus* influence.

SITE 44: New Mexico; Hidalgo County; 16.1 km (linear) S and 2.4 km (linear) E of Road Forks; 1310 m elev. AMNH R-127170–79; FT 738–47; sample 19. Habitat: mixed *Prosopis* and *Larrea* desertscrub on sandy soil with *Gutierrezia*, *Koeberlinea*, and very few *Yucca*. Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids. This off-road site cost three tires and a lot of effort on a hot, sunny day, as we reached it by turning SE onto the gravel road at the junction 15.4 km (by Hwy 80) S of Road Forks, going 1.4 km SE on this gravel road, then leaving the road and driving another 1.8 km eastward, somewhat zigzag, cross-country through the creosote.

SITE 45: New Mexico; Hidalgo County; 14.5 km (linear) S and 3.2 km (linear) E of Road Forks; 1310 m elev. AMNH R-128413–22; FT 1047–56; sample 31. Habitat: mixed *Larrea* and *Prosopis* desertscrub on sandy soil with gravel, *Gutierrezia*, and very few *Koeberlinea*. Specimens are *C. t. punctilinealis* × *C. t. marmoratus* hybrids.

SITE 46: New Mexico; Hidalgo County; 15.8 km (linear) S and 5.0 km (linear) E of Road Forks; 1300 m elev. AMNH R-127200–09; FT 827–36; sample 20. Habitat: *Prosopis* grassland with *Gutierrezia*, fourwing saltbush (*Atriplex canescens*), *Koeberlinea*, and graythorn (*Condalia lycioides*). Specimens are largely *C. t. marmoratus*. We reached this site as follows: take NM Hwy 9 to Animas; turn N onto NM Hwy 338 in Animas; go 18.4 km, then turn W onto a gravel road; go 1.9 km to the end of the road, through the gate at the fence, then continue another 0.8 km W as directly as possible, cross-country.

SITE 47: New Mexico; Hidalgo County; Table Top Mountain, 19.3 km (linear) S and 12.1 km (linear) E of Road Forks; 1325 m elev. AMNH R-127088–97; FT 812–21; sample 12. Habitat: *Larrea* desertscrub with *Gutierrezia*, *Flourensia*, and very few *Prosopis*. Specimens are *C. t. marmoratus*.

SITE 48: New Mexico: Hidalgo County; 1.0 km (by NM Hwy 9) E and 15.4 km (by gravel

road) N of Animas; 1370 m elev. AMNH R-127068–77; FT 688–97; sample 11. Habitat: mixed *Larrea* and *Flourensia* desertscrub with *Gutierrezia*, *Ephedra*, scattered *Yucca*, and widely scattered cholla and prickly pear (*Opuntia*) cacti. Specimens are *C. t. marmoratus*. This was published as sample 10 in Dessauer and Cole (1991).

In addition, we examined proteins of one specimen of *Cnemidophorus neomexicanus* (AMNH R-133157) and one of *Cnemidophorus uniparens* (AMNH R-133250) from the following locality: New Mexico; Hidalgo County; 26.9 km (by NM Hwy 70) NW of Lordsburg; 1,310 m elev.

APPENDIX 2

Specimens Examined from Outside the Contact Region

The specimens are listed alphabetically by subspecies and then in numerical order of the collecting sites (fig. 1), with data and abbreviations presented as in appendix 1. Dates of collection are between 28 June 1982 and 19 July 1990. The specific date for each specimen is available from the AMNH. Virtually all of the following 78 specimens were used to examine the full suite of 36 gene loci analyzed electrophoretically.

Cnemidophorus tigris aethiops

SITE 63: MEXICO: Sonora; along the Rio Mayo, Navojoa. AMNH R-131431–32; FT 1675–76; sample 62.

SITE 64: MEXICO: Sonora; 6.5 km (by road) NE of Bahia de San Carlos. AMNH R-129164–65; FT 1097–98; sample 61. Also, MEXICO: Sonora; Bahia de San Carlos. AMNH R-131430; FT 1677; sample 61.

Cnemidophorus tigris marmoratus

SITE 52: New Mexico: Luna County; 2.9 km (by gravel road) S of Gage (30.6 km by Hwy I-10 E Deming). AMNH R-127120–29; FT 837–46; sample 8.

SITE 53: New Mexico: Dona Ana County; 3.9 km (by NM Hwy 26 and County Rd E4) SW of Hatch. AMNH R-127112–19; FT 555–62; sample 7.

SITE 54: New Mexico: Socorro County; 2.2 km (by road) W of San Antonio. AMNH R-131072–81; FT 1634–43; sample 33.

SITE 55: Laboratory-bred specimen that was not used.

Cnemidophorus tigris punctilinealis

SITE 49 (fig. 2): Arizona: Pima County; Huerfano Butte, Santa Rita Experimental Range, 43 km (linear) SSE of Tucson; 1140 m elev. AMNH R-127056–66; FT 655–64 and 666; sample 1.

SITE 50: Arizona: Cochise County; 5.6 km (linear) N and 1.6 km (linear) W of Portal. FT 1871–72; sample 23. Both specimens were frozen whole; there are no vouchers.

SITE 51: New Mexico: Hidalgo County; Antelope Pass, Peloncillo Mountains, W of Animas. FT 1935 and 1996–99 (Charles W. Painter, New Mexico Dept. Game and Fish numbers ESP 3338–41); sample 45.

SITE 56: Arizona: Pima County; Oak Flats Campground, 21.6 km (by AZ Hwy 60) WSW of Miami (Gila County); 1220 m elev. AMNH R-127067; FT 505; sample 60.

Cnemidophorus tigris septentrionalis

SITE 57: Arizona: Coconino County; 6.9 km (by AZ Hwy 264) SE of Moenkopi. AMNH R-136802–12; FT 2000–06 and 2011–14; sample 66.

SITE 58: Arizona: Coconino County; Salt Trail Canyon, Little Colorado River, 9.7 km upriver from the confluence with the Colorado River. AMNH R-136814–16; FT 2125–27; sample 50.

SITE 59: Arizona: Apache County; Many Farms. AMNH R-136796–800; FT 2015–18 and 2109; sample 68.

SITE 60: Utah: San Juan County; 1.9 km (by UT Hwy 163) N of Arizona state line. AMNH R-136786-88; FT 2010 and 2021–22; sample 67.

SITE 61: Utah: San Juan County; S side of the San Juan River, 5.8 km (by UT Hwy 163) E of Bluff. AMNH R-136789–95; FT 2102–08; sample 69.

SITE 62: Utah: Uinta County; Dinosaur National Monument. AMNH R-119557; HCD 3180; sample 64.

APPENDIX 3

Genetic and coloration hybrid index data for the 614 specimens of *Cnemidophorus tigris punctilinealis* (P) and *C. t. marmoratus* (M) from the contact region (details in appendix 1) plus sites 50 and 51 (appendix 2). See figures 3–5 for maps. For sex, F = female, M = male; HI = mean coloration hybrid index; mtDNA = mitochondrial DNA. For gene loci, allelic data are as follows: 0 = no data, 1 = *a*, 2 = *b*, 3 = *c*, 4 = *d*, and 5 = *e*. MDHP is the s locus. The following loci were invariant or included only rare alleles as variants (see table 22): ADH, G3PDH, LDH1, LDH2, sMDH, mMDH, mIDH, sSOD, mSOD, DDH, sAAT, AK, CK1, CK2, ALP, ESTD, PEPE, ADA, mACOH, MPI, PGM1, PGM2, and HB.

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
SITE 1 (*N* = 10)																
2076	F	0.04	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0103	0101	0202
2077	M	0.08	P	0202	0202	0202	0202	0202	0202	0202	0203	0203	0202	0203	0101	0202
2078	F	0.04	P	0202	0202	0202	0202	0202	0102	0202	0203	0101	0202	0303	0101	0202
2079	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0203	0202	0202	0303	0101	0202
2080	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0101	0202
2081	M	0.00	P	0202	0202	0202	0202	0203	0202	0202	0202	0203	0202	0203	0101	0202
2092	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0101	0202
2093	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0103	0101	0202
2094	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0101	0202
2095	F	0.04	P	0202	0202	0202	0202	0202	0202	0202	0202	0203	0202	0303	0102	0102
SITE 2 (*N* = 9)																
2026	F	0.21	M	0202	0202	0202	0202	0202	0202	0202	0202	0203	0202	0303	0101	0102
2049	M	0.12	P	0202	0202	0202	0202	0202	0102	0202	0203	0303	0202	0203	0103	0202
2050	M	0.08	P	0202	0102	0202	0202	0202	0102	0202	0202	0105	0202	0103	0101	0202
2051	F	0.17	M	0202	0202	0202	0202	0202	0102	0202	0203	0203	0202	0103	0101	0202
2052	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0103	0102	0102
2053	M	0.00	P	0202	0102	0202	0202	0202	0102	0202	0203	0101	0202	0203	0101	0202
2054	M	0.00	P	0202	0102	0202	0202	0202	0202	0202	0202	0305	0202	0202	0101	0101
2055	M	0.00	M	0102	0101	0202	0202	0202	0102	0202	0203	0102	0202	0203	0101	0202
2056	F	0.00	P	0202	0202	0202	0202	0202	0101	0202	0202	0203	0202	0203	0101	0202
SITE 3 (*N* = 20)																
2027	M	0.54	P	0102	0102	0202	0202	0202	0202	0202	0303	0103	0202	0103	0101	0101
2028	M	0.12	P	0102	0202	0202	0202	0202	0202	0202	0202	0202	0202	0303	0101	0102
2029	F	0.17	M	0202	0102	0202	0202	0202	0202	0202	0203	0102	0202	0103	0101	0000
2030	F	0.00	P	0202	0202	0202	0202	0202	0102	0203	0202	0101	0202	0303	0101	0102
2057	M	0.25	M	0202	0202	0202	0202	0202	0102	0202	0204	0102	0202	0203	0101	0202
2058	M	0.54	M	0202	0102	0202	0202	0202	0101	0202	0303	0103	0202	0103	0101	0202
2059	M	0.38	M	0101	0102	0202	0202	0202	0102	0202	0203	0204	0202	0102	0101	0202
2060	F	0.25	P	0202	0202	0202	0202	0202	0102	0202	0203	0101	0202	0303	0101	0102
2061	M	0.08	P	0202	0202	0202	0202	0202	0102	0202	0303	0203	0202	0203	0102	0101
2062	M	0.33	M	0202	0202	0202	0202	0202	0102	0202	0202	0305	0202	0202	0101	0101
6724	F	0.38	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0102	0202
6725	F	0.54	P	0202	0101	0102	0202	0202	0102	0202	0303	0303	0202	0202	0102	0101
6726	F	0.42	P	0102	0202	0202	0202	0202	0102	0202	0303	0102	0202	0303	0101	0102
6727	M	1.00	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0101
6736	F	0.29	M	0202	0202	0202	0202	0202	0102	0202	0202	0101	0202	0101	0101	0102
6737	M	0.62	M	0202	0102	0202	0202	0202	0202	0202	0303	0101	0202	0303	0101	0102
6738	M	0.71	M	0202	0102	0202	0202	0202	0102	0202	0203	0103	0202	0203	0101	0101
6739	M	0.33	M	0202	0102	0202	0202	0202	0202	0202	0202	0102	0202	0203	0101	0202
6740	F	0.29	P	0202	0102	0102	0202	0202	0101	0202	0202	0101	0202	0203	0101	0102
6741	F	0.25	P	0202	0102	0202	0202	0202	0101	0202	0202	0102	0202	0103	0101	0102
SITE 4 (*N* = 10)																
2031	M	0.58	M	0102	0101	0202	0202	0202	0102	0203	0303	0103	0202	0303	0101	0102
2032	F	0.58	M	0202	0101	0202	0202	0202	0102	0202	0303	0305	0202	0101	0101	0101

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
							SITE 4 (*continued*)									
2033	F	0.79	M	0102	0101	0202	0202	0202	0101	0202	0304	0203	0202	0103	0101	0101
2034	M	0.88	M	0202	0202	0202	0202	0202	0102	0202	0203	0103	0102	0101	0101	0101
2063	F	0.88	M	0101	0101	0202	0202	0202	0101	0202	0303	0203	0202	0103	0101	0101
2064	M	0.58	P	0101	0102	0202	0202	0202	0102	0202	0303	0305	0202	0101	0102	0101
2065	M	0.33	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0102
2066	F	0.62	M	0202	0101	0202	0202	0202	0102	0202	0304	0203	0102	0102	0101	0102
2067	M	0.33	M	0102	0102	0202	0202	0202	0102	0202	0203	0303	0202	0102	0102	0102
2068	F	0.50	M	0202	0101	0202	0202	0202	0102	0202	0303	0203	0202	0203	0101	0101
							SITE 5 (*N* = 10)									
2035	F	0.67	M	0102	0102	0202	0202	0203	0101	0202	0303	0303	0202	0202	0101	0101
2036	F	0.88	M	0101	0101	0202	0202	0202	0102	0202	0304	0303	0202	0101	0101	0102
2037	F	0.67	M	0102	0202	0202	0202	0202	0102	0202	0303	0303	0202	0102	0101	0101
2069	M	0.79	M	0102	0102	0202	0202	0202	0102	0203	0303	0103	0202	0102	0101	0202
2070	M	0.92	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0102
2071	F	1.00	M	0102	0101	0202	0202	0202	0101	0202	0303	0505	0202	0202	0101	0101
2072	M	0.96	M	0102	0101	0202	0202	0202	0101	0202	0303	0305	0202	0102	0101	0102
2073	F	0.58	M	0202	0101	0202	0202	0202	0102	0202	0303	0103	0202	0103	0101	0102
2074	F	0.58	M	0102	0101	0102	0202	0202	0102	0202	0203	0103	0202	0102	0101	0101
2075	F	0.79	M	0101	0102	0202	0202	0202	0102	0202	0303	0303	0202	0102	0101	0101
							SITE 6 (*N* = 10)									
2038	F	0.71	M	0102	0202	0202	0202	0202	0102	0202	0303	0305	0202	0202	0101	0101
2039	M	0.79	P	0202	0102	0202	0202	0202	0101	0202	0304	0105	0202	0102	0101	0101
2040	M	1.00	M	0202	0102	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
2041	F	0.83	M	0102	0101	0202	0202	0202	0101	0202	0303	0103	0202	0103	0101	0101
2042	M	0.92	M	0202	0102	0202	0202	0202	0101	0202	0303	0105	0202	0202	0101	0102
2043	M	0.96	M	0102	0101	0101	0202	0202	0101	0202	0303	0304	0202	0102	0101	0101
2044	F	0.75	P	0102	0102	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0101
2045	F	0.92	M	0101	0102	0202	0202	0202	0102	0202	0303	0303	0202	0103	0101	0101
2046	F	0.92	M	0202	0102	0202	0202	0202	0102	0202	0303	0305	0202	0102	0101	0102
2047	F	0.79	M	0202	0101	0202	0202	0203	0101	0202	0203	0303	0202	0202	0101	0101
							SITE 7 (*N* = 10)									
2082	M	0.54	M	0102	0102	0202	0202	0202	0101	0203	0303	0303	0202	0303	0101	0101
2083	M	0.96	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
2084	M	1.00	M	0101	0101	0202	0202	0202	0101	0202	0304	0303	0202	0202	0101	0101
2085	M	0.92	M	0202	0102	0202	0202	0202	0101	0202	0303	0303	0202	0103	0101	0101
2086	M	0.88	M	0102	0101	0202	0202	0202	0101	0203	0303	0305	0202	0102	0101	0101
2087	M	0.92	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0101
2088	M	0.83	M	0102	0101	0202	0202	0202	0101	0203	0303	0305	0202	0202	0101	0101
2089	M	0.92	M	0202	0101	0202	0202	0202	0101	0202	0303	0105	0202	0202	0101	0101
2090	F	0.92	M	0102	0102	0202	0202	0203	0101	0202	0303	0303	0202	0202	0101	0101
2091	F	0.96	M	0202	0101	0202	0202	0202	0101	0202	0304	0305	0202	0202	0101	0101
							SITE 8 (*N* = 2)									
2210	F	0.83	M	0102	0102	0202	0102	0202	0101	0202	0303	0303	0202	0102	0101	0101
6325	M	0.04	P	0202	0202	0202	0202	0202	0202	0202	0203	0101	0202	0203	0101	0202
							SITE 9 (*N* = 10)									
6330	M	0.12	P	0102	0202	0202	0202	0202	0101	0202	0202	0101	0202	0203	0101	0102
6331	F	0.04	P	0102	0202	0202	0202	0202	0101	0203	0202	0204	0202	0203	0101	0202

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
SITE 9 (*continued*)															
6332	M	0.00	P	0202	0202	0202	0202	0202	0101	0202	0203	0102	0202	0203	0102	0202
6333	F	0.25	P	0202	0202	0202	0202	0202	0101	0202	0202	0102	0102	0303	0101	0102
6334	M	0.08	P	0202	0202	0202	0202	0202	0101	0202	0202	0103	0202	0203	0101	0202
6335	F	0.25	P	0202	0202	0202	0202	0202	0101	0202	0202	0104	0102	0303	0101	0202
6336	F	0.29	P	0202	0202	0202	0202	0202	0102	0202	0203	0104	0202	0203	0102	0102
6337	M	0.67	P	0202	0202	0202	0202	0202	0101	0202	0303	0102	0202	0203	0101	0202
6338	M	0.12	P	0202	0202	0202	0202	0202	0102	0202	0202	0101	0202	0203	0101	0202
6339	F	0.25	M	0202	0202	0202	0202	0202	0101	0202	0202	0202	0202	0303	0101	0102
SITE 10 (*N* = 10)															
2136	F	0.00	P	0202	0202	0202	0203	0202	0102	0202	0202	0102	0202	0303	0101	0202
2141	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0204	0202	0303	0101	0101
2142	F	0.00	P	0202	0202	0202	0203	0202	0102	0202	0202	0204	0202	0303	0101	0202
2170	M	0.08	P	0202	0202	0202	0202	0202	0102	0202	0202	0405	0202	0303	0101	0202
2171	F	0.04	P	0202	0202	0202	0202	0202	0102	0202	0202	0204	0202	0303	0101	0202
2172	M	0.00	P	0202	0102	0202	0202	0202	0101	0202	0202	0203	0202	0303	0101	0102
2173	F	0.04	P	0202	0102	0202	0202	0202	0202	0202	0202	0304	0202	0303	0101	0202
2175	F	0.00	P	0202	0101	0202	0202	0202	0102	0202	0202	0203	0202	0303	0101	0202
2176	M	0.00	P	0202	0101	0202	0202	0202	0202	0202	0202	0204	0202	0303	0101	0202
6340	M	0.25	P	0202	0202	0202	0202	0202	0202	0202	0202	0405	0000	0303	0102	0202
SITE 11 (*N* = 10)															
6311	M	0.33	P	0202	0202	0202	0202	0202	0202	0202	0203	0102	0202	0303	0101	0202
6312	M	0.00	P	0202	0102	0202	0202	0202	0202	0202	0102	0105	0102	0303	0101	0202
6313	F	0.17	P	0202	0202	0202	0202	0202	0202	0202	0203	0101	0202	0103	0101	0202
6314	M	0.04	P	0202	0202	0202	0202	0202	0101	0202	0203	0205	0202	0203	0101	0202
6315	M	0.00	P	0202	0202	0202	0202	0202	0102	0202	0203	0102	0202	0203	0101	0202
6316	F	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0303	0101	0202
6317	M	0.04	P	0202	0202	0202	0202	0202	0102	0202	0202	0202	0202	0303	0101	0202
6318	F	0.25	P	0202	0202	0202	0102	0202	0202	0202	0202	0305	0202	0303	0101	0202
6319	F	0.04	P	0202	0102	0202	0202	0202	0202	0202	0202	0105	0202	0303	0101	0202
6326	M	0.12	P	0202	0202	0202	0203	0202	0101	0202	0202	0203	0202	0203	0101	0202
SITE 12 (*N* = 10)															
6353	M	0.00	P	0102	0202	0202	0202	0202	0102	0202	0203	0102	0202	0203	0101	0202
6354	F	0.00	—	0202	0202	0202	0202	0202	0202	0202	0202	0204	0202	0303	0101	0202
6355	M	0.33	P	0202	0202	0202	0102	0202	0202	0202	0203	0205	0202	0303	0101	0102
6357	F	0.04	P	0202	0202	0202	0202	0000	0202	0202	0202	0303	0202	0103	0101	0102
6359	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0203	0202	0202	0103	0101	0202
6360	M	0.04	P	0202	0202	0202	0202	0202	0202	0202	0203	0101	0202	0303	0101	0101
6361	M	0.17	P	0202	0202	0202	0202	0102	0202	0202	0203	0205	0202	0303	0101	0102
6362	M	0.92	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0203	0101	0101
6363	M	0.00	P	0202	0202	0202	0203	0202	0202	0202	0202	0102	0202	0303	0101	0202
6364	F	0.08	P	0202	0202	0202	0202	0202	0101	0202	0202	0102	0202	0303	0101	0102
SITE 13 (*N* = 10)															
6341	F	0.92	M	0202	0101	0202	0202	0202	0101	0203	0303	0303	0202	0102	0101	0101
6342	M	0.92	M	0102	0102	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
6343	F	1.00	M	0101	0101	0202	0202	0202	0101	0202	0203	0303	0202	0102	0101	0101
6344	M	0.83	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0103	0101
6345	F	1.00	M	0102	0102	0202	0202	0202	0102	0202	0303	0303	0202	0101	0101	0101
6346	M	0.71	M	0101	0102	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
						SITE 13 (continued)										
6348	M	1.00	M	0101	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
6349	M	0.92	M	0102	0102	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
6350	F	1.00	M	0101	0101	0202	0202	0202	0101	0202	0303	0304	0202	0203	0101	0101
6351	M	0.92	M	0102	0102	0202	0202	0202	0101	0202	0304	0303	0202	0101	0101	0101
						SITE 14 (N = 88)										
0282	M	—	M	0202	0101	0202	0202	0202	0102	0202	0303	0303	0202	0202	0101	0101
0283	M	—	M	0102	0101	0202	0202	0203	0101	0202	0303	0303	0202	0202	0101	0101
0284	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
0343	F	—	M	0202	0101	0202	0202	0202	0102	0203	0303	0303	0202	0102	0101	0101
0345	M	—	M	0101	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
0346	M	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0304	0202	0202	0101	0101
0347	M	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0203	0101	0101	0101
0348	F	—	M	0101	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
0516	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0000
0517	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0000
0518	M	—	M	0102	0102	0202	0202	0202	0101	0202	0303	0305	0202	0202	0101	0000
0519	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0102	0000
0520	M	—	M	0102	0101	0202	0202	0202	0102	0202	0303	0303	0202	0102	0101	0000
0521	F	—	M	0102	0102	0202	0202	0202	0101	0202	0304	0303	0202	0102	0101	0000
0522	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0305	0202	0202	0101	0000
0523	F	—	M	0102	0101	0202	0202	0202	0102	0202	0303	0303	0202	0202	0101	0000
0524	F	—	M	0202	0102	0202	0202	0202	0102	0202	0303	0304	0202	0102	0101	0000
0525	F	—	M	0202	0101	0202	0202	0202	0102	0202	0304	0303	0202	0101	0101	0000
0526	F	—	M	0202	0101	0202	0202	0202	0101	0203	0303	0203	0202	0202	0202	0000
0527	F	—	M	0202	0101	0202	0202	0202	0102	0202	0303	0304	0203	0102	0101	0000
0528	M	—	M	0102	0101	0202	0202	0202	0102	0202	0303	0303	0202	0102	0101	0000
0529	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0000
0530	M	—	M	0202	0102	0202	0202	0202	0102	0202	0303	0303	0202	0202	0102	0000
0544	M	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0102
0545	F	—	M	0102	0102	0202	0202	0202	0101	0203	0303	0303	0202	0101	0101	0101
0546	—	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
0547	—	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
0581	M	0.92	M	0102	0101	0202	0202	0202	0101	0202	0303	0304	0202	0202	0101	0101
0588	M	0.83	M	0202	0101	0202	0202	0202	0101	0202	0303	0203	0202	0101	0101	0101
0589	M	0.92	M	0101	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
0892	F	—	M	0202	0101	0202	0202	0202	0102	0202	0303	0303	0202	0102	0101	0102
0893	F	—	M	0101	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
0894	—	—	M	0101	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
0895	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1108	M	—	M	0101	0101	0202	0202	0202	0101	0202	0303	0304	0202	0102	0101	0101
1109	M	—	M	0202	0101	0202	0202	0202	0102	0202	0303	0305	0202	0101	0101	0101
1111	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0305	0202	0202	0101	0000
1121	F	—	M	0102	0101	0202	0202	0202	0101	0203	0303	0303	0202	0102	0101	0101
1122	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0505	0202	0102	0101	0101
1233	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0404	0202	0102	0101	0101
1501	M	—	M	0202	0101	0202	0202	0202	0101	0303	0303	0303	0202	0102	0101	0101
5603	—	—	M	0101	0101	0202	0202	0202	0101	0202	0303	0000	0202	0202	0101	0101
5604	—	—	M	0202	0101	0202	0202	0202	0101	0203	0303	0303	0202	0202	0000	0101
1718	F	—	M	0102	0101	0202	0202	0202	0102	0202	0303	0303	0202	0202	0102	0101
1719	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1720	M	—	M	0102	0101	0202	0202	0202	0101	0203	0303	0303	0202	0102	0101	0101

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
							SITE 14 (*continued*)									
1721	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1723	F	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1724	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0101
1725	M	—	M	0101	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1726	F	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1727	M	—	M	0102	0102	0202	0202	0202	0101	0202	0303	0304	0202	0202	0103	0101
1728	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1730	M	—	M	0102	0101	0202	0202	0202	0101	0202	0203	0304	0202	0202	0101	0101
1794	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0304	0202	0102	0102	0101
1796	F	—	M	0202	0101	0202	0202	0202	0101	0202	0304	0305	0202	0202	0101	0101
1797	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0203	0202	0202	0101	0101
1798	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1801	M	—	M	0102	0101	0000	0000	0202	0101	0202	0303	0303	0202	0202	0101	0101
1802	M	—	M	0102	0101	0102	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1803	M	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0304	0202	0202	0101	0101
1804	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1805	M	—	M	0102	0101	0202	0202	0202	0102	0202	0303	0203	0202	0202	0101	0101
1806	F	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0103	0101
1807	M	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0304	0202	0202	0101	0101
1808	M	—	M	0101	0101	0202	0202	0202	0101	0202	0404	0303	0202	0202	0101	0101
1809	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1810	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0203	0202	0101	0000
1811	M	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0102	0101
1812	M	—	M	0101	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1818	M	—	M	0202	0101	0202	0202	0202	0101	0202	0304	0303	0202	0202	0101	0101
1819	M	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0101
1820	F	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1821	M	—	M	0202	0101	0202	0202	0202	0102	0202	0303	0303	0202	0101	0101	0101
1822	F	—	M	0102	0101	0202	0202	0202	0102	0202	0303	0303	0202	0101	0101	0101
1823	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1824	F	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1840	M	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1841	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1842	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1843	M	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1844	M	—	M	0202	0102	0202	0202	0202	0101	0202	0303	0304	0202	0102	0101	0102
1845	M	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1846	M	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1847	F	—	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
1848	F	—	M	0202	0101	0202	0202	0202	0102	0202	0303	0303	0202	0202	0101	0101
1849	M	—	M	0202	0102	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1860	F	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0101
							SITE 15 (*N* = 3)									
0281	M	—	M	0102	0102	0202	0202	0202	0102	0202	0303	0303	0203	0102	0101	0101
0329	F	—	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
0344	F	—	—	0000	0101	0202	0202	0202	0101	0202	0203	0304	0000	0102	0000	0101
							SITE 16 (*N* = 10)									
6255	F	1.00	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0102
6256	M	0.88	M	0101	0101	0202	0202	0202	0101	0102	0303	0203	0202	0102	0102	0101
6258	F	0.96	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
							SITE 16 (*continued*)									
6259	F	0.92	M	0102	0101	0202	0202	0202	0101	0202	0304	0303	0202	0102	0101	0101
6260	F	0.96	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
6261	M	0.96	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0102	0101
6320	F	0.96	M	0102	0101	0102	0202	0202	0101	0202	0304	0303	0202	0102	0102	0101
6321	F	1.00	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
6322	F	0.92	M	0102	0101	0202	0202	0202	0101	0202	0303	0305	0202	0202	0101	0101
6323	F	1.00	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
							SITE 17 (*N* = 1)									
2152	M	1.00	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0103	0101	0101
							SITE 18 (*N* = 10)									
2153	M	1.00	M	0202	0202	0202	0202	0202	0101	0202	0303	0204	0202	0202	0101	0101
2154	F	0.54	M	0202	0202	0202	0202	0202	0101	0202	0303	0404	0202	0103	0101	0101
2165	F	0.71	M	0202	0202	0202	0202	0202	0101	0202	0303	0305	0202	0101	0101	0102
2177	M	0.96	M	0102	0101	0202	0202	0202	0102	0202	0203	0203	0202	0202	0101	0101
2178	M	0.33	M	0101	0102	0202	0202	0202	0101	0203	0203	0105	0202	0102	0101	0102
2179	F	0.75	M	0202	0101	0202	0202	0202	0202	0202	0202	0105	0202	0103	0102	0102
2180	M	0.96	M	0202	0102	0202	0202	0202	0101	0202	0303	0304	0202	0102	0101	0101
6324	F	0.75	M	0202	0202	0202	0202	0202	0101	0202	0303	0105	0000	0101	0102	0101
6327	F	0.96	M	0202	0101	0202	0202	0000	0101	0202	0303	0104	0202	0102	0102	0102
6328	M	0.83	M	0202	0102	0202	0202	0202	0101	0202	0303	0304	0000	0101	0101	0101
							SITE 19 (*N* = 10)									
2184	M	0.75	—	0202	0101	0202	0202	0202	0102	0202	0203	0305	0202	0203	0101	0101
2185	M	0.50	P	0202	0202	0202	0202	0202	0102	0202	0203	0305	0202	0102	0101	0102
2186	M	0.62	M	0202	0102	0202	0202	0202	0202	0202	0203	0203	0202	0103	0101	0101
2187	M	0.04	M	0202	0102	0202	0202	0202	0102	0202	0303	0203	0202	0103	0101	0202
2188	F	0.38	M	0202	0202	0202	0202	0202	0202	0202	0202	0202	0202	0203	0101	0202
2189	M	0.58	M	0202	0102	0202	0202	0202	0102	0202	0203	0203	0202	0303	0101	0102
2190	F	0.08	M	0202	0102	0202	0202	0202	0102	0202	0203	0203	0202	0303	0101	0102
2191	M	0.21	M	0202	0102	0202	0202	0202	0102	0202	0202	0102	0202	0303	0101	0202
2192	M	0.25	M	0202	0102	0202	0203	0202	0101	0202	0203	0101	0202	0303	0101	0101
2193	F	0.17	M	0202	0101	0102	0202	0202	0101	0202	0204	0102	0202	0203	0101	0102
							SITE 20 (*N* = 31)									
2194	M	0.00	P	0202	0202	0202	0202	0202	0202	0000	0202	0101	0202	0203	0102	0202
2195	M	0.00	P	0202	0202	0202	0202	0202	0202	0000	0202	0103	0202	0203	0101	0202
2196	M	0.00	P	0202	0202	0202	0202	0202	0202	0000	0202	0102	0101	0203	0203	0202
2197	F	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0105	0202	0203	0101	0202
2198	M	0.00	P	0202	0202	0202	0202	0202	0102	0202	0202	0105	0202	0203	0102	0202
2199	F	0.00	P	0202	0202	0202	0202	0202	0101	0202	0202	0102	0202	0102	0102	0102
2200	F	0.00	P	0202	0202	0202	0202	0202	0202	0000	0202	0103	0203	0203	0102	0202
2201	F	0.00	—	0202	0202	0202	0202	0202	0102	0202	0202	0203	0202	0303	0101	0202
2202	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0102	0202	0101	0202
2203	M	0.12	P	0202	0202	0202	0203	0202	0202	0202	0202	0103	0202	0303	0101	0202
6283	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0203	0101	0202	0303	0102	0202
6284	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0101	0202
6285	F	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0101	0202
6286	F	0.04	P	0202	0202	0202	0203	0202	0202	0202	0202	0101	0202	0303	0102	0202
6287	M	0.00	P	0202	0202	0202	0101	0202	0202	0202	0202	0101	0202	0203	0101	0202
6288	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0102	0202

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
							SITE 20 (*continued*)									
6289	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0203	0102	0202
6290	M	0.00	P	0202	0202	0202	0203	0202	0202	0202	0202	0101	0202	0203	0102	0202
6291	M	0.00	P	0202	0202	0102	0202	0202	0202	0202	0202	0101	0102	0303	0102	0202
6292	F	0.00	P	0202	0202	0102	0203	0203	0202	0202	0202	0101	0202	0203	0102	0202
6294	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0203	0101	0202	0303	0102	0000
6713	M	0.00	—	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0102	0202
6714	M	0.00	P	0202	0202	0202	0202	0202	0102	0202	0202	0102	0202	0303	0102	0202
6715	M	0.00	—	0202	0202	0202	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202
6716	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0203	0101	0202
6717	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0203	0101	0202
6718	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0203	0102	0303	0102	0202
6719	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0101	0202
6720	F	0.00	P	0202	0202	0202	0102	0202	0202	0202	0202	0102	0202	0203	0101	0202
6721	M	0.00	P	0202	0202	0202	0303	0202	0202	0202	0202	0101	0102	0203	0101	0202
6722	F	0.00	P	0202	0202	0202	0102	0202	0101	0202	0203	0102	0202	0303	0101	0202
							SITE 21 ($N = 10$)									
6262	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0303	0101	0202
6264	M	0.00	P	0202	0202	0102	0202	0202	0202	0202	0202	0204	0202	0202	0101	0202
6265	F	0.00	P	0202	0202	0202	0202	0202	0202	0102	0202	0102	0202	0203	0102	0202
6266	F	0.00	P	0202	0202	0202	0202	0202	0102	0202	0202	0102	0202	0303	0101	0202
6267	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0304	0202	0303	0101	0202
6268	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0303	0101	0202
6269	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0204	0202	0303	0102	0202
6270	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0203	0102	0202
6271	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0303	0101	0202
6272	F	0.00	P	0202	0000	0202	0202	0203	0202	0202	0202	0101	0202	0203	0102	0202
							SITE 22 ($N = 10$)									
1019	M	0.00	P	0202	0202	0102	0202	0202	0202	0202	0203	0202	0202	0303	0102	0202
1020	F	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0203	0102	0202
1021	F	0.08	P	0202	0202	0202	0202	0202	0202	0202	0202	0105	0202	0203	0101	0202
1022	F	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0101	0102
1023	F	0.08	P	0202	0202	0202	0202	0202	0102	0202	0202	0101	0202	0203	0101	0202
1024	F	0.00	P	0202	0102	0202	0202	0202	0102	0202	0202	0101	0202	0303	0101	0202
1025	M	0.00	P	0202	0202	0202	0202	0202	0102	0202	0202	0203	0202	0202	0101	0202
1026	F	0.08	P	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0203	0101	0202
1027	M	0.17	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0203	0101	0202
1028	M	0.00	P	0202	0202	0202	0202	0000	0202	0202	0202	0101	0202	0203	0102	0202
							SITE 23 ($N = 10$)									
0968	F	0.17	P	0202	0202	0202	0202	0202	0102	0202	0203	0203	0202	0303	0101	0202
0969	F	0.25	P	0202	0102	0202	0202	0202	0202	0202	0202	0105	0202	0303	0101	0202
0970	F	0.08	M	0202	0102	0202	0203	0202	0202	0202	0202	0202	0202	0202	0101	0102
0971	M	0.08	P	0202	0202	0202	0202	0202	0202	0202	0202	0303	0202	0203	0101	0202
0972	M	0.50	P	0202	0102	0202	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202
0973	M	0.00	P	0202	0102	0202	0202	0202	0202	0202	0202	0205	0202	0202	0101	0202
0974	M	0.00	M	0202	0102	0202	0202	0202	0101	0202	0202	0104	0202	0203	0101	0202
0975	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0203	0202	0203	0101	0202
0976	F	0.08	M	0202	0102	0202	0203	0202	0202	0202	0203	0104	0202	0203	0102	0202
0977	F	0.50	M	0202	0202	0202	0202	0202	0202	0202	0203	0102	0202	0202	0101	0202

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
							SITE 24 (*N* = 10)									
1009	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0202	0202	0203	0101	0202
1010	F	0.42	M	0202	0202	0202	0203	0202	0202	0202	0203	0303	0202	0203	0101	0102
1011	F	0.08	P	0202	0102	0202	0202	0202	0202	0202	0202	0202	0202	0103	0101	0202
1012	M	0.25	M	0202	0202	0202	0202	0202	0202	0202	0203	0202	0202	0203	0102	0202
1013	M	0.08	P	0202	0102	0202	0202	0202	0202	0202	0203	0202	0202	0203	0102	0202
1014	F	0.42	M	0202	0202	0202	0202	0202	0102	0202	0203	0202	0202	0303	0101	0202
1015	M	0.17	P	0202	0102	0202	0202	0202	0102	0202	0203	0202	0202	0203	0102	0202
1016	M	0.33	P	0202	0202	0202	0202	0202	0102	0202	0202	0202	0202	0202	0102	0102
1017	F	0.17	M	0202	0202	0202	0202	0202	0202	0202	0202	0202	0203	0203	0101	0202
1018	F	0.25	P	0202	0202	0202	0202	0000	0202	0202	0202	0202	0202	0202	0101	0102
							SITE 25 (*N* = 10)									
0978	F	0.17	M	0202	0101	0202	0202	0202	0101	0202	0203	0303	0202	0102	0202	0102
0979	F	0.33	M	0202	0102	0202	0202	0202	0101	0202	0203	0303	0202	0203	0101	0102
0980	M	0.17	M	0202	0202	0202	0202	0202	0102	0202	0204	0103	0202	0102	0101	0202
0981	M	0.75	M	0202	0101	0202	0202	0202	0102	0202	0204	0103	0202	0203	0101	0102
0982	F	0.42	P	0202	0202	0202	0202	0202	0101	0202	0203	0103	0202	0103	0101	0101
0983	F	0.17	M	0202	0102	0102	0202	0202	0202	0202	0202	0203	0102	0202	0101	0202
0984	M	0.25	P	0202	0102	0202	0202	0202	0202	0202	0202	0202	0202	0203	0101	0202
0985	M	0.33	P	0202	0202	0202	0202	0202	0202	0202	0203	0305	0202	0203	0102	0202
0986	M	0.50	M	0202	0102	0202	0202	0202	0102	0202	0203	0203	0202	0103	0101	0202
0987	M	0.33	P	0202	0102	0202	0202	0202	0102	0202	0203	0303	0202	0303	0101	0202
							SITE 26 (*N* = 31)									
1617	M	0.21	P	0202	0102	0202	0202	0202	0102	0203	0203	0203	0202	0303	0101	0202
1618	M	0.67	M	0202	0101	0202	0202	0202	0101	0202	0203	0103	0202	0203	0102	0101
1619	M	0.96	M	0202	0101	0202	0202	0202	0101	0202	0303	0203	0202	0102	0101	0101
1620	M	0.75	M	0202	0101	0202	0202	0202	0102	0202	0303	0103	0202	0202	0101	0102
1621	M	0.25	M	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0203	0202	0202
1622	M	0.50	M	0102	0202	0202	0202	0202	0101	0202	0203	0202	0202	0203	0101	0101
1623	M	0.04	M	0202	0202	0202	0202	0202	0102	0202	0203	0102	0202	0203	0101	0102
1624	M	0.04	P	0202	0102	0202	0202	0202	0202	0202	0203	0103	0202	0303	0101	0202
1631	F	1.00	P	0102	0102	0202	0202	0202	0102	0202	0203	0203	0202	0203	0101	0202
1632	F	0.54	M	0202	0202	0202	0202	0202	0102	0202	0203	0202	0202	0103	0101	0202
6252	M	0.33	M	0202	0101	0202	0202	0202	0102	0203	0203	0305	0202	0303	0101	0000
6273	F	0.50	P	0202	0101	0102	0202	0202	0102	0202	0203	0303	0202	0102	0101	0102
6274	F	0.25	P	0202	0102	0202	0202	0202	0102	0202	0204	0102	0202	0203	0102	0202
6275	F	0.62	P	0202	0101	0202	0202	0202	0102	0202	0303	0304	0202	0202	0101	0102
6276	M	0.21	M	0202	0101	0202	0202	0202	0102	0202	0204	0305	0202	0203	0101	0102
6277	F	0.50	M	0102	0102	0202	0202	0202	0101	0202	0203	0103	0202	0101	0101	0102
6278	M	0.54	P	0202	0101	0202	0202	0202	0102	0202	0203	0303	0202	0102	0101	0101
6279	M	0.75	M	0202	0101	0202	0202	0202	0101	0202	0304	0303	0202	0102	0101	0101
6280	F	0.62	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0102
6281	M	1.00	M	0202	0101	0202	0202	0202	0202	0203	0203	0103	0202	0102	0101	0102
6282	M	0.75	P	0202	0102	0202	0202	0202	0102	0202	0203	0103	0202	0102	0101	0101
6728	F	0.29	P	0202	0202	0202	0202	0202	0102	0202	0202	0203	0202	0203	0101	0102
6729	F	0.12	M	0102	0102	0202	0202	0202	0101	0202	0202	0102	0202	0203	0102	0102
6730	F	0.88	M	0202	0102	0202	0202	0202	0102	0202	0203	0203	0202	0203	0101	0102
6731	F	0.29	M	0202	0102	0202	0202	0202	0101	0202	0203	0303	0202	0203	0101	0102
6732	M	0.88	M	0202	0102	0202	0202	0202	0101	0202	0303	0202	0202	0102	0101	0101
6733	M	0.29	P	0202	0202	0202	0202	0202	0102	0203	0202	0103	0202	0303	0101	0102
6734	F	0.12	M	0202	0102	0202	0202	0202	0202	0203	0303	0303	0202	0102	0101	0202

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
							SITE 26 (*continued*)									
6735	M	0.25	M	0202	0102	0202	0202	0202	0101	0202	0202	0102	0202	0203	0102	0202
6743	M	0.42	M	0202	0102	0202	0202	0202	0102	0203	0203	0203	0202	0103	0102	0202
6744	M	0.29	M	0202	0101	0202	0202	0202	0102	0203	0202	0103	0202	0202	0102	0101
							SITE 27 (*N* = 10)									
0998	M	0.67	P	0202	0101	0202	0202	0202	0101	0202	0202	0305	0202	0102	0101	0102
0999	F	0.58	M	0202	0102	0202	0202	0202	0101	0203	0303	0203	0202	0101	0102	0102
1000	M	0.58	M	0202	0202	0202	0202	0202	0102	0203	0303	0102	0202	0202	0101	0101
1001	M	0.42	M	0202	0102	0202	0202	0202	0101	0202	0203	0202	0202	0203	0101	0102
1002	M	0.42	M	0202	0102	0202	0202	0202	0101	0202	0203	0303	0202	0103	0101	0102
1003	F	0.58	M	0202	0202	0202	0202	0202	0101	0203	0203	0303	0202	0202	0101	0202
1004	F	0.33	M	0202	0102	0202	0202	0202	0202	0202	0203	0103	0202	0303	0102	0101
1005	M	0.75	P	0202	0102	0202	0202	0202	0102	0202	0203	0303	0202	0103	0101	0102
1006	M	0.58	M	0102	0102	0202	0202	0202	0102	0203	0304	0303	0202	0102	0101	0102
1007	M	0.25	M	0202	0102	0202	0202	0202	0101	0202	0203	0303	0202	0102	0102	0102
							SITE 28 (*N* = 12)									
0988	M	0.83	M	0202	0202	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0202
0989	F	1.00	M	0102	0101	0202	0202	0202	0102	0202	0203	0303	0202	0203	0101	0102
0990	F	0.75	M	0202	0102	0202	0202	0202	0101	0202	0303	0303	0202	0202	0102	0101
0991	M	0.75	M	0202	0102	0202	0202	0202	0101	0202	0303	0103	0202	0102	0101	0101
0992	F	0.67	M	0202	0202	0202	0202	0202	0102	0202	0303	0203	0202	0202	0101	0202
0993	M	0.75	P	0102	0101	0202	0202	0202	0101	0202	0303	0102	0202	0202	0101	0101
0994	F	0.67	M	0102	0102	0202	0202	0202	0101	0202	0303	0305	0202	0102	0101	0101
0995	M	0.83	M	0202	0102	0202	0202	0202	0101	0202	0203	0303	0202	0102	0101	0102
0996	M	0.92	M	0202	0101	0202	0202	0202	0102	0202	0303	0305	0202	0101	0101	0101
997a	F	0.83	M	0202	0101	0202	0202	0202	0101	0202	0303	0103	0202	0103	0101	0102
997b	—	—	M	0202	0101	0202	0202	0202	0101	0000	0303	0303	0202	0103	0101	0102
997c	M	—	M	0102	0000	0202	0202	0202	0102	0000	0303	0203	0202	0303	0101	0202
							SITE 29 (*N* = 30)									
2159	M	0.88	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
2160	M	0.92	M	0202	0101	0202	0202	0202	0101	0202	0303	0203	0202	0101	0101	0101
2161	M	0.62	M	0202	0101	0202	0202	0202	0101	0202	0303	0305	0202	0202	0101	0101
2162	M	1.00	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0101
2163	M	1.00	M	0101	0101	0202	0202	0202	0101	0202	0203	0303	0202	0202	0101	0101
2164	F	1.00	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
2166	F	1.00	M	0202	0101	0202	0202	0202	0101	0202	0203	0303	0202	0102	0101	0101
2167	F	0.92	M	0202	0101	0202	0202	0202	0101	0202	0303	0203	0202	0102	0101	0101
2168	F	1.00	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
2169	M	0.83	M	0101	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
6295	F	0.96	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
6296	M	0.96	M	0202	0101	0202	0202	0202	0101	0202	0303	0304	0202	0102	0101	0101
6297	F	0.92	M	0202	0101	0202	0202	0202	0101	0203	0303	0303	0202	0202	0101	0101
6298	F	0.96	M	0202	0101	0202	0202	0202	0101	0202	0303	0304	0202	0101	0101	0101
6299	M	0.92	M	0102	0102	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
6300	F	1.00	M	0102	0101	0202	0202	0203	0101	0202	0303	0303	0202	0102	0101	0101
6301	F	0.92	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
6302	M	0.96	M	0101	0101	0202	0202	0202	0101	0203	0303	0303	0202	0102	0101	0101
6303	M	0.92	M	0202	0101	0202	0202	0202	0101	0202	0304	0303	0202	0102	0101	0101
6304	M	0.88	M	0102	0101	0202	0202	0202	0101	0202	0304	0304	0202	0202	0101	0101
6745	F	0.92	M	0101	0102	0202	0202	0102	0101	0203	0303	0303	0202	0202	0101	0101

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
						SITE 29 (*continued*)										
6746	F	0.79	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0102	0101
6747	F	0.96	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
6748	M	0.88	M	0102	0102	0202	0202	0202	0102	0000	0303	0303	0202	0101	0101	0101
6749	M	0.75	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
6750	F	0.92	M	0102	0101	0202	0202	0000	0101	0203	0303	0303	0202	0102	0101	0101
6751	M	0.88	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
6752	M	1.00	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0102	0101
6753	M	0.88	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
6754	F	1.00	M	0202	0101	0202	0202	0202	0101	0202	0304	0304	0202	0101	0000	0101
						SITE 30 (*N* = 10)										
1032	F	1.00	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1033	M	0.92	M	0202	0102	0202	0202	0202	0101	0202	0303	0303	0202	0203	0101	0102
1034	M	0.92	M	0102	0102	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0102
1035	F	0.67	M	0202	0101	0202	0202	0202	0102	0203	0303	0203	0202	0103	0101	0101
1036	M	1.00	M	0102	0102	0202	0202	0202	0101	0202	0303	0303	0202	0103	0102	0101
1037	M	0.92	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
1038	M	0.83	M	0202	0101	0202	0202	0202	0101	0203	0303	0305	0202	0102	0101	0101
1039	F	0.58	M	0202	0101	0202	0202	0202	0101	0202	0303	0203	0202	0101	0101	0101
1040	M	0.92	M	0102	0101	0202	0202	0202	0102	0202	0203	0103	0202	0103	0101	0101
1041	F	0.92	M	0202	0102	0102	0202	0202	0101	0202	0203	0303	0202	0103	0101	0102
						SITE 31 (*N* = 10)										
0768	M	0.83	M	0102	0102	0202	0202	0202	0101	0202	0303	0105	0202	0102	0101	0101
0769	F	0.75	M	0202	0101	0202	0202	0202	0102	0202	0303	0303	0202	0101	0101	0101
0770	M	1.00	M	0202	0101	0202	0202	0202	0101	0202	0204	0303	0202	0101	0101	0101
0771	F	1.00	M	0202	0101	0202	0202	0202	0101	0202	0203	0303	0202	0202	0101	0101
0772	M	0.92	M	0102	0101	0202	0202	0202	0101	0203	0303	0303	0202	0102	0101	0102
0773	M	1.00	M	0102	0101	0202	0202	0202	0101	0203	0303	0303	0202	0103	0101	0101
0774	M	0.92	M	0102	0102	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0101
0775	M	0.83	M	0202	0101	0202	0202	0202	0102	0202	0303	0203	0202	0102	0101	0101
0776	M	1.00	M	0202	0101	0202	0202	0202	0101	0202	0303	0305	0203	0202	0101	0101
0777	F	1.00	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0101
						SITE 32 (*N* = 10)										
0778	M	1.00	M	0202	0101	0202	0202	0202	0101	0202	0303	0203	0202	0101	0101	0101
0779	M	0.75	M	0202	0101	0202	0202	0202	0101	0203	0303	0305	0202	0202	0101	0101
0780	F	0.83	M	0101	0202	0202	0202	0202	0101	0203	0303	0303	0202	0101	0101	0102
0781	F	0.75	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
0782	M	0.83	M	0202	0101	0202	0202	0202	0101	0203	0304	0303	0202	0101	0101	0101
0783	F	1.00	M	0102	0102	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0102
0784	M	0.67	M	0102	0101	0202	0202	0202	0101	0203	0303	0303	0202	0101	0101	0101
0785	F	0.92	M	0202	0101	0202	0202	0202	0101	0202	0303	0305	0202	0103	0101	0101
0786	F	0.67	M	0202	0101	0202	0202	0202	0101	0202	0303	0305	0202	0202	0101	0101
0787	F	0.71	M	0202	0102	0202	0202	0202	0101	0303	0303	0303	0202	0101	0101	0101
						SITE 33 (*N* = 10)										
0698	M	0.58	M	0202	0101	0202	0202	0202	0101	0203	0303	0303	0202	0101	0101	0101
0699	M	0.71	M	0102	0102	0202	0202	0202	0101	0303	0303	0101	0202	0103	0101	0101
0700	F	0.67	M	0202	0101	0202	0202	0202	0102	0202	0303	0303	0202	0202	0101	0101
0701	M	0.62	M	0202	0102	0202	0202	0202	0203	0203	0203	0303	0202	0101	0101	0101
0702	F	0.83	M	0102	0202	0202	0202	0202	0101	0303	0303	0303	0202	0202	0101	0101

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
							SITE 33 (*continued*)									
0703	M	0.46	M	0202	0102	0202	0202	0202	0101	0203	0203	0104	0202	0203	0101	0101
0704	F	1.00	M	0202	0101	0202	0202	0202	0102	0202	0203	0303	0202	0102	0101	0101
0705	F	0.75	M	0202	0202	0202	0202	0202	0101	0202	0303	0203	0202	0202	0101	0102
0706	M	0.83	M	0102	0101	0202	0202	0202	0202	0202	0203	0303	0202	0102	0101	0102
0707	M	1.00	M	0202	0101	0202	0202	0202	0101	0203	0303	0103	0202	0203	0101	0101
							SITE 34 ($N = 10$)									
0748	M	0.25	P	0202	0102	0202	0202	0202	0202	0202	0202	0105	0202	0202	0101	0202
0749	M	0.12	P	0202	0202	0202	0202	0202	0101	0202	0203	0202	0102	0202	0101	0202
0750	M	0.17	P	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0103	0102	0202
0751	M	0.00	P	0102	0102	0202	0202	0202	0202	0202	0303	0203	0202	0303	0101	0202
0752	F	0.00	M	0202	0202	0202	0202	0203	0202	0203	0203	0303	0202	0103	0101	0202
0753	F	0.04	P	0202	0202	0202	0202	0202	0202	0202	0202	0203	0102	0203	0101	0102
0754	F	0.00	M	0202	0202	0202	0203	0203	0102	0202	0202	0102	0202	0303	0101	0102
0755	F	0.00	P	0202	0202	0202	0203	0202	0202	0202	0202	0103	0202	0303	0102	0202
0756	M	0.08	P	0202	0202	0202	0202	0202	0102	0202	0203	0101	0202	0203	0101	0102
0757	M	0.00	P	0102	0102	0202	0203	0202	0202	0202	0202	0101	0202	0303	0101	0202
							SITE 35 ($N = 10$)									
0788	M	0.00	P	0202	0202	0202	0202	0202	0101	0202	0202	0102	0202	0203	0101	0202
0789	F	0.00	M	0202	0102	0202	0202	0202	0102	0202	0202	0205	0202	0303	0101	0202
0790	M	0.04	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0203	0101	0202
0791	M	0.17	P	0202	0202	0202	0202	0202	0202	0202	0202	0202	0202	0203	0101	0202
0792	F	0.00	P	0202	0202	0202	0203	0202	0102	0202	0303	0103	0202	0303	0101	0102
0793	M	0.00	M	0202	0202	0202	0202	0202	0102	0202	0202	0101	0202	0103	0101	0102
0794	M	0.00	P	0202	0102	0202	0202	0202	0101	0202	0202	0103	0202	0303	0101	0202
0795	F	0.00	P	0202	0202	0202	0202	0202	0102	0202	0303	0203	0202	0203	0101	0202
0796	M	0.08	M	0202	0202	0202	0202	0202	0102	0202	0202	0101	0202	0303	0202	0202
0797	F	0.00	P	0202	0102	0202	0202	0202	0101	0202	0203	0102	0202	0203	0101	0202
							SITE 36 ($N = 10$)									
0679	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0203	0101	0202
0680	M	0.00	P	0202	0202	0202	0203	0202	0202	0202	0202	0101	0202	0303	0102	0202
0681	M	0.00	P	0202	0102	0202	0203	0202	0202	0202	0202	0101	0102	0203	0102	0202
0682	M	0.00	P	0202	0202	0102	0303	0202	0102	0202	0202	0101	0102	0303	0102	0202
0683	M	0.00	P	0202	0202	0202	0202	0203	0202	0202	0202	0101	0202	0303	0102	0202
0684	M	0.00	P	0202	0202	0202	0202	0203	0202	0202	0202	0203	0102	0303	0101	0202
0685	F	0.00	P	0202	0202	0202	0203	0203	0102	0202	0202	0102	0202	0203	0101	0202
0686	M	0.00	P	0202	0202	0202	0202	0203	0202	0202	0202	0101	0202	0303	0101	0202
0687	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0303	0101	0202
6365	M	0.00	P	0202	0202	0202	0202	0202	0102	0202	0202	0101	0202	0203	0101	0202
							SITE 37 ($N = 10$)									
0798	F	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0303	0101	0202
0799	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0102	0202
0800	M	0.00	P	0202	0202	0102	0202	0202	0202	0202	0202	0102	0202	0303	0102	0202
0801	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0303	0102	0202
0802	F	0.00	P	0202	0102	0202	0202	0202	0202	0202	0202	0102	0202	0303	0101	0202
0803	F	0.08	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0102	0303	0101	0202
0804	M	0.00	P	0202	0202	0202	0203	0202	0202	0202	0202	0101	0202	0303	0101	0202
0805	M	0.00	P	0202	0202	0202	0203	0202	0202	0202	0202	0102	0203	0303	0101	0202
0806	M	0.04	P	0202	0202	0202	0202	0202	0102	0202	0202	0103	0202	0203	0101	0202
0807	F	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0303	0102	0202

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
							SITE 38 (*N* = 10)									
0728	M	0.00	P	0202	0202	0202	0203	0202	0202	0202	0202	0104	0102	0303	0101	0202
0729	F	0.00	P	0202	0202	0202	0202	0202	0102	0202	0202	0105	0202	0303	0102	0202
0730	F	0.04	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0102	0202
0731	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0203	0101	0202
0732	M	0.00	P	0202	0202	0202	0202	0202	0101	0202	0202	0102	0102	0303	0101	0202
0733	M	0.00	P	0202	0202	0202	0102	0202	0202	0202	0202	0202	0202	0303	0101	0202
0734	M	0.04	P	0202	0202	0202	0202	0202	0102	0101	0202	0104	0202	0303	0102	0202
0735	F	0.04	P	0202	0202	0202	0202	0102	0202	0202	0202	0102	0202	0202	0101	0202
0736	F	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0303	0101	0202
0737	F	0.00	P	0202	0202	0202	0202	0102	0102	0202	0202	0103	0202	0303	0101	0202
							SITE 39 (*N* = 10)									
0758	M	0.04	P	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0203	0101	0202
0759	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0303	0102	0202
0760	M	0.04	P	0202	0202	0102	0203	0202	0202	0202	0202	0303	0202	0202	0101	0202
0761	M	0.00	P	0202	0202	0202	0203	0202	0102	0202	0202	0103	0202	0303	0101	0202
0762	F	0.08	P	0202	0202	0202	0202	0202	0102	0202	0202	0203	0202	0303	0102	0202
0763	F	0.12	P	0202	0102	0202	0202	0202	0101	0202	0202	0204	0202	0303	0101	0202
0764	M	0.08	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0203	0101	0202
0765	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0203	0202	0203	0102	0202
0766	M	0.00	P	0202	0202	0202	0202	0202	0102	0202	0202	0103	0202	0203	0101	0202
0767	F	0.00	P	0202	0202	0202	0203	0102	0202	0202	0202	0103	0202	0203	0102	0202
							SITE 40 (*N* = 10)									
0718	F	0.04	P	0202	0202	0202	0202	0000	0202	0202	0202	0103	0202	0203	0102	0202
0719	M	0.00	P	0202	0202	0102	0202	0202	0202	0202	0202	0104	0202	0303	0101	0202
0720	M	0.21	P	0202	0102	0202	0202	0202	0202	0202	0202	0203	0202	0203	0101	0202
0721	F	—	P	0202	0202	0202	0202	0202	0102	0202	0202	0202	0202	0203	0102	0202
0722	M	0.00	P	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0203	0101	0202
0723	F	0.33	M	0102	0202	0202	0203	0202	0102	0202	0202	0203	0102	0303	0101	0202
0724	F	0.00	P	0202	0102	0202	0203	0000	0202	0202	0202	0101	0202	0303	0101	0102
0725	M	0.42	M	0202	0102	0202	0202	0202	0102	0202	0203	0103	0202	0203	0102	0102
0726	M	0.50	P	0202	0102	0202	0202	0202	0101	0202	0202	0102	0202	0202	0101	0202
0727	M	0.25	P	0202	0102	0202	0202	0202	0202	0203	0203	0104	0202	0103	0101	0202
							SITE 41 (*N* = 10)									
0854	M	0.38	P	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0303	0101	0102
0855	M	0.42	M	0202	0202	0202	0202	0202	0102	0202	0303	0203	0202	0203	0101	0102
0856	M	0.17	P	0202	0101	0202	0202	0202	0102	0202	0202	0303	0202	0103	0101	0102
0857	F	0.17	M	0202	0202	0202	0202	0202	0101	0202	0303	0103	0202	0303	0101	0202
0928	M	0.58	P	0202	0102	0202	0202	0202	0202	0202	0202	0101	0202	0303	0101	0202
0965	M	0.08	P	0202	0202	0202	0202	0202	0202	0202	0202	0303	0202	0203	0102	0202
0966	M	0.08	P	0202	0202	0202	0202	0202	0101	0202	0202	0103	0202	0203	0101	0202
0967	M	0.50	P	0202	0102	0202	0202	0202	0101	0202	0203	0303	0202	0202	0101	0102
1045	F	0.17	P	0202	0102	0102	0202	0202	0102	0202	0202	0103	0202	0203	0101	0102
1046	F	0.50	M	0102	0101	0202	0202	0202	0101	0202	0203	0303	0202	0101	0101	0101
							SITE 42 (*N* = 10)									
0668	M	0.04	M	0102	0102	0202	0202	0202	0101	0202	0202	0103	0202	0202	0101	0102
0669	F	0.08	M	0202	0102	0202	0202	0202	0101	0202	0203	0202	0202	0303	0202	0102
0670	F	0.54	M	0202	0202	0202	0202	0202	0101	0202	0202	0101	0202	0303	0101	0202
0671	M	0.29	M	0202	0101	0202	0202	0202	0101	0202	0203	0103	0102	0203	0101	0102

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
							SITE 42 (*continued*)									
0672	M	0.62	P	0102	0102	0202	0202	0202	0101	0102	0202	0101	0202	0202	0101	0202
0673	M	1.00	P	0202	0102	0202	0203	0202	0101	0202	0203	0303	0202	0103	0101	0202
0674	F	—	M	0202	0102	0202	0203	0202	0102	0202	0203	0102	0202	0102	0101	0101
0675	M	0.88	M	0202	0102	0202	0202	0202	0101	0303	0202	0101	0202	0202	0101	0102
0676	F	—	M	0202	0202	0202	0202	0202	0101	0202	0202	0305	0202	0103	0101	0101
0677	M	0.21	M	0202	0102	0202	0202	0202	0102	0202	0202	0102	0202	0303	0101	0102
							SITE 43 ($N = 10$)									
0708	F	0.00	P	0202	0102	0202	0202	0202	0102	0202	0203	0102	0202	0203	0101	0102
0709	M	0.50	M	0202	0102	0202	0202	0202	0102	0202	0203	0101	0202	0102	0101	0202
0710	M	0.33	M	0202	0202	0202	0202	0202	0202	0202	0202	0103	0202	0203	0202	0202
0711	F	0.00	P	0202	0202	0202	0202	0202	0202	0202	0203	0102	0202	0203	0101	0202
0712	F	0.00	M	0202	0202	0202	0202	0202	0202	0202	0202	0303	0202	0203	0101	0202
0713	F	0.12	P	0202	0102	0202	0202	0202	0202	0202	0203	0101	0202	0303	0102	0202
0714	F	0.04	P	0202	0102	0202	0202	0202	0102	0202	0202	0101	0202	0203	0101	0102
0715	M	0.17	M	0202	0102	0202	0202	0202	0202	0202	0203	0205	0202	0101	0101	0102
0716	M	0.21	P	0202	0102	0202	0202	0202	0202	0203	0303	0101	0202	0102	0101	0202
0717	M	0.25	P	0202	0102	0202	0202	0202	0202	0203	0203	0103	0202	0103	0101	0102
							SITE 44 ($N = 10$)									
0738	M	0.50	M	0202	0101	0202	0202	0202	0101	0202	0202	0103	0202	0303	0101	0102
0739	F	0.25	M	0102	0101	0202	0202	0202	0101	0202	0202	0202	0202	0103	0102	0102
0740	M	0.50	M	0202	0102	0102	0202	0202	0101	0202	0203	0303	0202	0102	0102	0102
0741	M	0.42	P	0202	0101	0202	0202	0202	0102	0202	0303	0103	0202	0303	0101	0102
0742	M	0.67	M	0102	0101	0202	0202	0202	0101	0202	0303	0103	0202	0102	0101	0101
0743	F	1.00	M	0202	0202	0202	0202	0203	0101	0202	0303	0303	0202	0101	0101	0102
0744	M	0.42	M	0202	0102	0202	0202	0202	0102	0202	0203	0103	0202	0203	0202	0102
0745	M	0.67	M	0202	0101	0202	0202	0203	0101	0202	0203	0102	0202	0303	0101	0202
0746	M	0.58	M	0102	0202	0202	0202	0202	0101	0202	0203	0303	0202	0203	0102	0102
0747	M	0.54	M	0202	0202	0202	0202	0202	0101	0202	0203	0203	0202	0102	0101	0101
							SITE 45 ($N = 10$)									
1047	F	0.83	M	0202	0102	0202	0202	0202	0101	0203	0303	0404	0202	0103	0101	0101
1048	F	0.67	M	0102	0101	0202	0202	0202	0101	0203	0203	0404	0202	0203	0101	0101
1049	M	1.00	M	0202	0102	0202	0202	0202	0101	0202	0303	0101	0202	0202	0101	0102
1050	F	0.67	M	0202	0101	0202	0202	0202	0102	0203	0303	0305	0202	0101	0101	0101
1051	F	0.67	M	0202	0102	0202	0202	0202	0101	0202	0303	0404	0202	0102	0101	0101
1052	M	0.92	M	0102	0101	0202	0202	0203	0101	0202	0203	0404	0202	0103	0101	0101
1053	F	0.75	M	0202	0101	0202	0202	0202	0102	0203	0202	0404	0202	0101	0101	0102
1054	M	0.75	M	0102	0102	0202	0202	0203	0102	0202	0303	0104	0202	0102	0101	0101
1055	F	0.50	M	0202	0101	0202	0202	0202	0101	0202	0303	0104	0202	0102	0101	0101
1056	M	0.67	M	0102	0101	0202	0202	0202	0101	0202	0203	0404	0202	0102	0101	0101
							SITE 46 ($N = 10$)									
0827	M	0.83	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0101	0101	0101
0828	M	1.00	M	0202	0101	0202	0202	0202	0101	0202	0203	0103	0202	0103	0101	0102
0829	F	1.00	M	0202	0101	0202	0202	0202	0102	0203	0303	0303	0202	0102	0101	0101
0830	M	0.75	M	0102	0101	0202	0202	0202	0102	0203	0304	0303	0202	0202	0101	0101
0831	F	0.67	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0203	0101	0101
0832	F	0.92	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
0833	F	0.83	M	0102	0102	0202	0202	0202	0101	0202	0303	0205	0202	0102	0101	0101
0834	F	0.67	M	0202	0101	0202	0202	0202	0101	0203	0203	0303	0202	0203	0101	0102
0835	M	1.00	M	0202	0101	0202	0202	0202	0101	0202	0303	0505	0202	0202	0101	0102
0836	M	0.83	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101

APPENDIX 3—(*Continued*)

FT#	Sex	HI	mtDNA	IDDH	MDHP	sIDH	mAAT	EST1	EST2	PEPA	PEPB	PEPD	sACOH	GPI	PGM3	TF
							SITE 47 (*N* = 10)									
0812	F	0.92	M	0101	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
0813	M	0.92	M	0101	0101	0202	0202	0202	0101	0202	0304	0303	0202	0102	0101	0101
0814	M	0.92	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0202	0101	0101
0815	M	0.83	M	0202	0101	0202	0202	0202	0101	0202	0303	0103	0202	0102	0101	0101
0816	M	0.92	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
0817	F	0.92	M	0202	0101	0202	0202	0202	0101	0202	0304	0303	0202	0102	0101	0101
0818	F	1.00	M	0202	0101	0202	0202	0202	0101	0202	0203	0303	0202	0102	0101	0101
0819	F	1.00	M	0102	0101	0202	0202	0202	0101	0202	0304	0303	0202	0102	0101	0101
0820	M	1.00	M	0102	0101	0202	0202	0202	0101	0202	0203	0303	0202	0102	0101	0101
0821	M	1.00	M	0102	0101	0202	0202	0202	0101	0202	0304	0303	0202	0102	0101	0101
							SITE 48 (*N* = 10)									
0688	M	0.75	P	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
0689	F	0.92	M	0102	0101	0202	0202	0202	0101	0203	0303	0103	0202	0102	0101	0101
0690	F	0.96	M	0102	0101	0202	0202	0202	0102	0202	0303	0303	0202	0101	0101	0101
0691	M	1.00	M	0202	0101	0202	0202	0202	0101	0202	0404	0303	0202	0101	0101	0101
0692	F	1.00	M	0202	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
0693	F	0.92	M	0101	0101	0202	0202	0202	0102	0202	0304	0303	0202	0102	0101	0101
0694	F	0.96	M	0202	0101	0202	0202	0202	0102	0202	0303	0303	0202	0202	0101	0101
0695	F	0.92	M	0102	0101	0202	0202	0202	0101	0202	0303	0303	0202	0102	0101	0101
0696	F	1.00	M	0102	0101	0202	0202	0202	0101	0202	0203	0303	0202	0102	0101	0101
0697	M	0.79	M	0102	0101	0202	0202	0202	0102	0202	0303	0102	0202	0102	0101	0101
							SITE 50 (*N* = 2)									
1871	M	—	P	0202	0202	0202	0202	0202	0202	0202	0202	0101	0202	0303	0101	0202
1872	M	—	P	0202	0202	0202	0202	0202	0202	0202	0202	0102	0202	0303	0101	0202
							SITE 51 (*N* = 5)									
1935	M	—	P	0202	0102	0202	0202	0202	0202	0202	0202	0303	0202	0303	0101	0202
1996	F	—	P	0202	0102	0202	0202	0202	0102	0202	0203	0101	0202	0203	0101	0202
1997	M	—	P	0202	0202	0202	0203	0202	0102	0202	0202	0203	0202	0303	0102	0202
1998	F	—	P	0202	0202	0202	0203	0202	0202	0202	0202	0203	0202	0303	0101	0202
1999	M	—	P	0202	0102	0202	0202	0202	0202	0202	0202	0103	0202	0303	0101	0202

APPENDIX 4

Contingency Tables Used for Calculating Cytonuclear Disequilibrium Indices

mtDNA	Genotypes			Total	mtDNA	Genotypes			Total

NORTHERN HYBRID REGION[a]

	sMDHP ($N = 49$)					PEPB ($N = 49$)			
	aa	*ab*	*bb*			*cc*	*bc*	*bb*	
M	15 (0.306)	10 (0.204)	7 (0.143)	32 (0.653)	M	19 (0.388)	9 (0.184)	4 (0.082)	32 (0.653)
P	1 (0.020)	7 (0.143)	9 (0.184)	17 (0.347)	P	5 (0.102)	3 (0.061)	9 (0.184)	17 (0.347)
Total	16 (0.326)	17 (0.347)	16 (0.327)		Total	24 (0.490)	12 (0.245)	13 (0.266)	
	EST2 ($N = 49$)					TF ($N = 48$)			
	aa	*ab*	*bb*			*aa*	*ab*	*bb*	
M	13 (0.265)	14 (0.286)	5 (0.102)	32 (0.653)	M	13 (0.271)	11 (0.229)	7 (0.146)	31 (0.646)
P	3 (0.061)	9 (0.184)	5 (0.102)	17 (0.347)	P	5 (0.104)	7 (0.146)	5 (0.104)	17 (0.354)
Total	16 (0.326)	23 (0.470)	10 (0.204)		Total	18 (0.375)	18 (0.375)	12 (0.250)	

CENTRAL HYBRID REGION[b]

	sMDHP ($N = 70$)					PEPB ($N = 70$)			
	aa	*ab*	*bb*			*cc*	*bc*	*bb*	
M	15 (0.214)	26 (0.371)	12 (0.171)	53 (0.757)	M	17 (0.243)	27 (0.386)	9 (0.129)	53 (0.757)
P	4 (0.057)	8 (0.114)	5 (0.071)	17 (0.243)	P	1 (0.014)	13 (0.186)	3 (0.043)	17 (0.243)
Total	19 (0.271)	34 (0.485)	17 (0.242)		Total	18 (0.257)	40 (0.572)	12 (0.172)	
	EST2 ($N = 70$)					TF ($N = 69$)			
	aa	*ab*	*bb*			*aa*	*ab*	*bb*	
M	27 (0.386)	18 (0.257)	8 (0.114)	53 (0.757)	M	16 (0.232)	24 (0.348)	12 (0.174)	52 (0.754)
P	2 (0.029)	12 (0.171)	3 (0.043)	17 (0.243)	P	3 (0.043)	7 (0.101)	7 (0.101)	17 (0.246)
Total	29 (0.415)	30 (0.428)	11 (0.157)		Total	19 (0.275)	31 (0.449)	19 (0.275)	

SOUTHERN HYBRID REGION[c]

	sMDHP ($N = 70$)					PEPB ($N = 70$)			
	aa	*ab*	*bb*			*cc*	*bc*	*bb*	
M	11 (0.157)	13 (0.186)	15 (0.214)	39 (0.557)	M	10 (0.143)	16 (0.229)	13 (0.186)	39 (0.557)
P	2 (0.029)	15 (0.214)	14 (0.200)	31 (0.443)	P	5 (0.071)	9 (0.129)	17 (0.243)	31 (0.443)
Total	13 (0.186)	28 (0.400)	29 (0.414)		Total	15 (0.214)	25 (0.358)	30 (0.429)	
	EST2 ($N = 69$)					TF ($N = 70$)			
	aa	*ab*	*bb*			*aa*	*ab*	*bb*	
M	22 (0.319)	11 (0.159)	5 (0.072)	38 (0.551)	M	13 (0.186)	17 (0.243)	9 (0.129)	39 (0.557)
P	8 (0.116)	8 (0.116)	15 (0.217)	31 (0.449)	P	0 (0.000)	11 (0.157)	20 (0.286)	31 (0.443)
Total	30 (0.435)	19 (0.275)	20 (0.289)		Total	13 (0.186)	28 (0.400)	29 (0.415)	

APPENDIX 4—(*Continued*)

mtDNA	Genotypes			Total	mtDNA	Genotypes			Total

ENTIRE REGION-I
(freq. *marmoratus* alleles between 0.2 and 0.8)[d]

	sMDHP (*N* = 189)					PEPB (*N* = 188)			
	aa	*ab*	*bb*			*cc*	*bc*	*bb*	
M	41 (0.217)	49 (0.259)	34 (0.180)	124 (0.656)	M	46 (0.245)	51 (0.271)	26 (0.138)	123 (0.654)
P	7 (0.037)	30 (0.159)	28 (0.148)	65 (0.344)	P	11 (0.059)	25 (0.133)	29 (0.154)	65 (0.346)
Total	48 (0.254)	79 (0.418)	62 (0.328)		Total	57 (0.304)	76 (0.404)	55 (0.292)	
	EST2 (*N* = 188)					TF (*N* = 187)			
	aa	*ab*	*bb*			*aa*	*ab*	*bb*	
M	62 (0.330)	43 (0.229)	18 (0.096)	123 (0.654)	M	42 (0.225)	52 (0.278)	28 (0.150)	122 (0.652)
P	13 (0.069)	29 (0.154)	23 (0.122)	65 (0.346)	P	8 (0.043)	25 (0.134)	32 (0.171)	65 (0.348)
Total	75 (0.399)	72 (0.383)	41 (0.218)		Total	50 (0.268)	77 (0.412)	60 (0.321)	

ENTIRE REGION-II
(freq. *marmoratus* alleles between 0.4 and 0.6)[e]

	sMDHP (*N* = 97)					PEPB (*N* = 100)			
	aa	*ab*	*bb*			*cc*	*bc*	*bb*	
M	18 (0.186)	36 (0.371)	16 (0.165)	70 (0.722)	M	13 (0.130)	35 (0.350)	17 (0.170)	65 (0.650)
P	3 (0.031)	13 (0.134)	11 (0.113)	27 (0.278)	P	6 (0.060)	18 (0.180)	11 (0.110)	35 (0.350)
Total	21 (0.217)	49 (0.505)	27 (0.278)		Total	19 (0.190)	53 (0.530)	28 (0.280)	
	EST2 (*N* = 100)					TF (*N* = 98)			
	aa	*ab*	*bb*			*aa*	*ab*	*bb*	
M	35 (0.350)	25 (0.250)	10 (0.100)	70 (0.700)	M	17 (0.173)	33 (0.337)	18 (0.184)	68 (0.694)
P	6 (0.060)	18 (0.180)	6 (0.060)	30 (0.300)	P	6 (0.061)	14 (0.143)	10 (0.102)	30 (0.306)
Total	41 (0.410)	43 (0.430)	16 (0.160)		Total	23 (0.235)	47 (0.480)	28 (0.286)	

[a] Pooled data for sites 2 through 5 with mean frequencies of *marmoratus* alleles at the diagnostic loci between 0.2 and 0.8.

[b] Pooled data for sites 18, 19, 25, 26, and 27 with frequencies of *marmoratus* alleles at the diagnostic loci between 0.2 and 0.8.

[c] Pooled data for sites 33 through 35 and 41 through 44 with frequencies of *marmoratus* alleles at diagnostic loci between 0.2 and 0.8.

[d] Pooled data for all sites listed in the footnotes above.

[e] Pooled data from sites 3, 19, 25, 26, 27, 42 and 44, in each of which the mean frequencies of the *marmoratus* alleles at the diagnostic loci are between 0.4 and 0.6.